Morphological and ecological studies on helminth parasites of British shrews.

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Morphological and Ecological Studies on Helminth Parasites of British Shrews.

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Thesis submitted for the degree of Doctor of Philosophy, August 1992

"..whatever you do, do it all for the glory of God."

St. Paul's First Letter to the Corinthians

Chapter 10, verse 31 (The Holy Bible)

Abstract

The morphology and ecology of the helminth parasites of the common and pygmy shrew were studied from three sites in Berkshire. Mark-release-recapture indicated that juvenile shrews were born in the summer and a significant proportion died in late autumn along with all adults. The population remained relatively stable until the next generation were born.

29 helminth species were recovered from 129 *Sorex araneus* and 77 *S.minutus*. The cestodes *Hymenolepis infirma, H.jacutensis, H.prolifer* and *H.schaldybini,* the nematode *Stefanskostrongylus soricis* and the digenean, *Opisthioglyphe sobolevi* are new records for Britain and *Sorex minutus* is a new host for *Eucoleus kutori* (Nematoda). The taxonomy and morphology of the helminths is discussed.

Egg output by intestinal helminths showed no diurnal pattern and there was no relationship between egg production rate and number of mature worms.

Cysticerci of *Hymenolepis schaldybini* were recovered for the first time from *Anthobium unicolor* (Coleoptera, Staphylinidae) and metacercariae of *Brachylaemus sp.* (probably *B.fulvus*) from *Vitrina pellucida* and *Oxychilus helveticus* (Gastropoda).

Prevalences and intensities of infection were influenced by habitat and host age. Seasonal parasite abundance was linked with variation in intermediate host availability. Differences in the helminth fauna of the two shrews were likely to be due to dietary differences. All helminth species were overdispersed in the host population.

The helminth species exhibited niche separation allowing higher worm burdens to be supported than if all helminths were adapted to the same niche.

No serious pathogenic effects were observed in the shrew and helminths were thought unlikely to have an impact on the host population dynamics. It is suggested that old age exacerbated by sociological and other factors is responsible for the death of the adult shrews during the autumn, while sociological factors associated with territoriality are thought to play a major part in juvenile mortality.

Acknowledgements

I would like to thank my supervisors Dr J.S.Churchfield and Dr J.W.Lewis for their advice and encouragement.

I would also like to acknowledge the assistance given by Dr B.W.Ferry in the identification of the flora at the study sites and Dr D.T.Bilton, Dr R.B.Angus and Mr J.Hollier for their help with the identification of invertebrates.

I am also grateful to the many students who assisted with the intensive trapping programmes in Windsor Great Park.

Trapping was carried out by kind permission of the Crown Estates Department whom I thank for their co-operation, I am especially grateful to Mr J.Stubbs.

The help of Miss A.Giller, Miss M.Goddard and Dr J.Kubecka in translation of some of the references and the assistance of Miss J.Skeldon with some of the figures is also very much appreciated.

I would like to thank the academic and technical staff of the Biology Departments of Royal Holloway & Bedford New College and King's College for their advice, encouragement and assistance. I am also grateful to the staff of the Laboratoire des Vers, Muséum d'Histoire Naturelle, Paris for allowing me to spend a week there studying techniques for the examination of nematodes. The co-operation of the staff of the Parasitic Worms section of the Natural History Museum, London and the Parasitology Institute of the Commonwealth Agricultural Bureau is also gratefully acknowledged.

I am very grateful to my parents for their support and encouragement during the course of my university studies. I am also grateful for the hospitality of Bill and Helene Hopkins.

Finally, I would like to thank the many friends who have encouraged me during the course of my studies.

This research project was funded by a postgraduate award from the Natural Environment Research Council.

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General Introduction

General Introduction

Shrews (Soricidae) are ubiquitous small mammals found throughout most of the world. Three species are present in mainland Britain: the common shrew, *Sorex araneus* Linnaeus 1758, the pygmy shrew, *S.minutus* Linnaeus 1766 and the water shrew, *Neomys fodiens* (Schreber, 1777) Oldfield 1898. The water shrew is present throughout Britain, but its distribution is rather localised and it is less abundant than the other two species. The present study therefore concentrated on the common and pygmy shrews.

Shrews belong to the order Insectivora, unlike most other similarly sized small mammals found in Britain such as the wood mouse, *Apodemus sylvaticus* (Linnaeus, 1758), the field vole *Microtus agrestis* (Linnaeus, 1761) and the bank vole *Clethrionomys glareolus* (Schreber, 1780) which are members of the order Rodentia. This taxonomic difference is reflected in the diet which in rodents comprises a high percentage of vegetable matter while in shrews it is restricted almost entirely to invertebrate prey such as beetles (Coleoptera), earthworms (Lumbricidae), slugs and snails (Gastropoda) and spiders (Araneae).

The invertebrate diet of shrews predisposes them to infection by helminth parasites. Helminth parasites in small mammals comprise four major taxonomic groups: the cestodes or tapeworms (Class Cestoda), the flukes (Class Digenea), the nematodes or roundworms (Class Nematoda) and the acanthocephalans or spiny-headed worms (Class Acanthocephala). Almost all of the cestodes, digeneans and acanthocephalans, as well as many of the nematodes, require one or more invertebrate intermediate hosts for the development of their larval stages in order to complete their life cycles. The vertebrate definitive host (in which the parasites are sexually mature) becomes infected either as a result of direct penetration of their tegument by larval stages or by ingestion of infective stages, the latter being the more usual route, especially in the case of helminths of terrestrial vertebrates.

Thus, the shrew will become infected with helminth parasites through its food and is likely to harbour a more diverse helminth fauna than the other sympatric small mammals. This has been confirmed by previous studies of British small mammals (Sharpe, 1964; Lewis 1964, 1968, 1987).

Despite the larger number of helminth species found in shrews than in small rodents the helminth fauna of shrews has received less attention in the literature. This may in part be due to the lower population densities of shrews compared with other small mammals. Previous studies of the helminth fauna of British shrews (Thomas, 1953; James, 1954; Lewis, 1964, 1968) were not comprehensive, generally involving a study of a small number of hosts and/or an incomplete examination of the host organs.

More detailed studies of the helminth faunas of *Sorex araneus* and *S.minutus* have been carried out in the rest of Europe (Soltys, 1952, 1954; Prokopic, 1959; Vaucher, 1971; Mas Coma & Gallego, 1975). However further work is required on the morphology and taxonomy of many of the species described.

Thus, the present study aimed to provide a comprehensive list of the helminths found in British *S.araneus* and *S.minutus* and to add to the current understanding of the morphology and taxonomy of these species (Chapter 3).

The helminth faunas of *S.araneus* and *S.minutus* were studied both by autopsy of shrews (Chapter 3) and by analysis of faecal samples (Chapter 4). Autopsy allows the precise identification and enumeration of the helminths present in the host at the time of its death, but the removal of the host from the population is likely to disturb both the host and parasite populations. Information on the helminth parasites present may be obtained without killing the host, by examining faecal samples for the presence of helminth eggs. Only adult parasites (adult females in the case of nematodes and acanthocephalans which are dioecious) may be detected using this technique, but the advantage of faecal analysis is that the course of infection in an individual animal may be followed over a period of time.

Faecal analysis techniques have generally been used either for studying human helminths (Stoll, 1923; Lane, 1924, 1925; Stoll & Hausheer, 1926) or those of laboratory rodents (Kerboeuf, 1982, 1985; Dunn & Keymer, 1986). An objective of the present study was to develop a suitable technique for the identification and enumeration of helminth eggs in the faeces of shrews and to investigate the effectiveness of the number of eggs per gramme of faeces as an indication of the number of mature worms present in the host individual.

Analysis of faeces from shrews maintained in the laboratory could also be used

to provide further information on the biology of the helminths by measuring their fecundity and diurnal changes in the rate of egg output.

Although intermediate hosts of some species of helminths present in shrews have been identified, no intermediate hosts have yet been found for several of the species and it is also likely that the intermediate hosts discovered to date do not comprise a complete list for the helminth species concerned. In the present study, therefore, it was proposed to examine invertebrates obtained from the study sites for the presence of helminth larvae.

Knowledge of the identity and biology of intermediate hosts of shrew parasites is important in the investigation of the population dynamics of the parasites. Seasonal variations in the abundance of intermediate hosts have been postulated to explain seasonal changes in the helminth faunas of *S.araneus* and *S.minutus* (Kisielewska, 1961; Lewis, 1968). Changes in helminth abundance have in turn been postulated as responsible for changes in the population dynamics of *S.araneus* (Borowski & Dehnel, 1952; Kisielewska, 1961; Buckner, 1969).

The population cycles of shrews have attracted the attention of naturalists for at least the last hundred years. Adams (1910, 1913) was the first to provide scientific evidence that *S.araneus* was an annual. By plotting head and body lengths against time he showed that there were two distinct size classes (adults and juveniles) present in summer, but that only the juveniles (or sub-adults) were present during the winter. Brambell (1935) and Brambell & Hall (1936) demonstrated that overwintering *S.araneus* and *S.minutus* were always sexually immature sub-adults with no signs of previous sexual activity.

Mark-release-recapture studies by Michielsen (1966), Pernetta (1977) and Churchfield (1979, 1980, 1984) provided more detailed information on the population dynamics of *S.araneus* and *S.minutus*. Overwintering sub-adults become sexually mature in March/April (Churchfield, 1990). Juveniles are born in April/May after a gestation period of about twenty days for *S.araneus* (Searle, 1984) and slightly longer for *S.minutus* (Churchfield, 1990) and weaned twenty-three days later (Searle, 1984). Two or possibly three litters may be born each year (Michielsen, 1966; Pernetta, 1977). The population is swelled by the influx of juveniles, reaching a peak around August/September. The summer peak in

numbers is followed by a rapid decline in population size during October/November, the so-called "autumnal epidemic" (Adams, 1913) involving the death of all the adult shrews and a significant proportion of the juveniles. The size of the population remains fairly stable during the winter and spring before increasing rapidly during the following summer.

Mortality due to helminth parasites has been postulated as one of several possible factors responsible for the "autumnal epidemic". In order to investigate their potential to exert a regulatory effect on the host population, an understanding of the ecology of the parasites is required. This involves investigations of their life-cycles to determine the identity of the intermediate hosts and the means by which they become infected as well as the availability of intermediate hosts relative to habitat and time of year. Ecological studies also concern the distribution of the parasite population within the host population and the implications of this for parasite transmission and host mortality. Thus, behavioural or hormonal differences between individuals of different sex or age might render one category of hosts more susceptible to parasitism.

The frequency distribution of parasites in their host will affect the number of hosts which are infected with potentially lethal worm burdens. Although large worm burdens may in theory be pathogenic (Anderson & May, 1978 and May & Anderson, 1978), in practice worm burdens may not normally reach pathogenic levels. Competition between parasites in a host individual (density-dependent mechanisms) may prevent the establishment of lethal levels of parasitism (Keymer, 1982).

In the present study three main experimental field communities of *S.araneus* and *S.minutus* were studied in order to investigate the role of helminths in population regulation. The Silwood Park populations of *S.araneus* and *S.minutus* acted as models of the host population changes taking place at the other two sites. Shrews at Silwood were studied by means of a mark-release-recapture method and no individuals were removed from the population.

Seasonal changes in worm burden were monitored by analysis of faecal samples from Silwood and by autopsies of successive samples of shrews from the other two sites, Cranbourne Chase and Lipper Pond, which also yielded helminth

material for morphological and taxonomic studies.

Chapter 2
Population Cycles in *Sorex araneus* and *Sorex minutus*.

Introduction

The Annual Breeding Cycle in *Sorex araneus* and *S.minutus*

Population changes in shrews have long been a source of interest to naturalists. As noted by Adams (1913), earlier workers believed the large numbers of shrews found dead in October/November to have been killed by the "autumnal epidemic" supposed to be a disease or parasite. Adams' own theory (Adams 1910, 1913) was that the death of the shrews was due to old age. He noticed that from December until spring all specimens of *Sorex araneus* and *S.minutus* found were not sexually mature; the genital organs of both sexes began to enlarge in February attaining a large size in May and June. He also noticed that the tails and feet of winter specimens were in good condition, but that adult shrews had lost most of the hair from their tails and feet by the summer. By plotting head and body lengths against time he demonstrated two distinct size classes in the summer, but only one corresponding to the juvenile size class in winter. He therefore concluded that all shrews found during the winter were juveniles and not adults in which the genitalia had atrophied and the tails had grown hairy.

Adams (1913) also recorded that shrews trapped during the winter had teeth which were less worn than those of mature shrews in the summer. This observation was backed up by a study carried out by Crowcroft (1956) on *S.araneus* in Wytham Wood near Oxford. He used the height of the lower canine teeth as an index of age, checking his tooth wear index for wild shrews against the tooth wear of captive-reared shrews. The dimensions, tooth wear and reproductive condition of shrews in successive samples throughout the year provided evidence that *S.araneus* 'does not normally breed in the year of birth and dies before the end of the following year.' Additional evidence for the autumnal death of the adults was provided by the fact that pellets from the tawny owl, *Strix aluco*, contained no remains of adult shrews after October.

A detailed study of the changes in reproductive condition in *S.araneus* was carried out by Brambell (1935) who examined 577 males and 487 females. The ovaries of overwintering females and testes of overwintering males showed no sign of previous sexual activity, the adults usually all dying by November. He

found no evidence of shrews reaching sexual maturity in their first summer.

Grainger and Fairley (1978) carried out a detailed study of *S.minutus* in Ireland where it is the only species of shrew present. Their data on tooth wear and changes in head and body length showed similar trends to those mentioned above for *S.araneus*. Using the criteria employed by Brambell (1935) and Brambell and Hall (1936) they showed that no sexually mature shrews were found in winter and that there was no evidence of either males or females attaining maturity in their first summer.

Hence both *S.araneus* and *S.minutus* are annuals born during the summer, overwintering as immature sub-adults to breed during the following summer before dying off in the autumn. Exceptions to this general rule have been found, but they are extremely rare. Pucek (1960) found that 0.9% of female *S.araneus* and 5% of female *S.minutus* reached sexual maturity in their first summer. Borowski and Dehnel (1952) found seven *S.araneus* out of five generations and one *S.minutus* out of four generations reached their second winter.

Annual Changes in Population Size and Structure

Research carried out by Michielsen (1966) in Holland, Pernetta (1977) and Churchfield (1979, 1980, 1984) in England involving mark-release-recapture methods allowed the population changes in shrews to be examined in greater detail. In addition to following the fluctuations in population size using actual numbers of captures, these authors also plotted survivorship curves showing how the mortality rates of shrews varied at different times of the year.

Overwintering sub-adults reach sexual maturity in March/April (Churchfield, 1990). After a gestation period of about twenty days for *S.araneus* (Searle, 1984) and slightly longer for *S.minutus* (Churchfield, 1990) the juveniles are born and appear in the trappable population when weaned twenty-three days later (Searle, 1984). Michielsen (1966) recorded that juvenile *S.araneus* and *S.minutus* were first caught at the end of May/beginning of June. Pernetta (1977) reported the first appearance of juvenile *S.minutus* to have occurred in April and that of *S.araneus* in May. Churchfield (1990) stated that juvenile shrews first appear in early May.

Pernetta (1977) used an improved tooth wear index involving four

measurements of the lower canine teeth rather than the single one used by Crowcroft (1956). Using data produced by this method he proposed that female *S.araneus* have two periods of synchronous litter production, the first cohort of juveniles being born in May/June and the second in August/September.

The size of the population increases rapidly to a summer peak as the juveniles are recruited into it. Towards the end of the summer the population size begins to decline as adults die off. A further decrease in number of shrews is caused by the disappearance of a significant proportion of the juveniles between July and October. Pernetta (1977) and Churchfield observed a marked reduction (up to 50%) in the number of juvenile *S.araneus* in the first two months of life and suggested that this was due to mortality or possibly emigration. Michielsen observed a less marked disappearance of juvenile *S.araneus* and attributed it to dispersal rather than mortality. All three authors agreed that the winter mortality of *S.araneus* is fairly low and most subadults alive at the beginning of the winter survive to breed the following summer, dying out more or less simultaneously at the end of the breeding season.

The survival curves obtained by Michielsen (1966) and Pernetta (1977) suggest that significant mortality of *S.minutus* occurs among resident individuals rather than at the dispersal phase as in *S.araneus*, *S.minutus* appearing to have a greater winter and spring mortality rate; Michielsen proposed that this was due to *S.minutus* being more vulnerable to aerial predators and the impact of the weather.

The life histories and nature of the population cycles in *S.araneus* and *S.minutus* are therefore fairly well documented. However, as discussed below, there is much less certainty about the factors responsible for producing the observed population changes, especially the marked decrease in numbers of juveniles during the autumn months. Several authors, for example Buckner (1969) consider parasites to be a major cause of mortality during this period. One of the aims of the present study was to investigate the role of helminth parasites in the "autumnal epidemic".

Since there is considerable variation in yearly capture of shrews (Mezhzherin, 1960), timing of reproduction and of annual population fluctuations, it is essential

that any attempt to examine the effect of parasites on the shrew population should be accompanied by a comprehensive study of the host population dynamics.

In the present study two methods were used for investigating the population cycles of shrews and the changes in their parasite communities.

The first method involved monitoring a population of shrews by regular live-trapping, marking and releasing. The helminth parasites present in this population were monitored by identifying and counting the parasite eggs present in the faeces of known individual shrews collected from Longworth traps. The advantage of this method was that the shrew population was subjected to the minimum possible disturbance since no shrews were removed, and was therefore likely to be behaving naturally. However, the disadvantage was that (for reasons discussed in Chapter 4) it is not possible adequately to study a helminth community solely from host faecal samples.

It was therefore necessary to use a second method of studying the host and parasite population changes. This involved removing successive samples of shrews from a given area so that the helminths parasitising these shrews could be thoroughly examined. The disadvantage of this method was that the population structure was being artificially altered by the removal of shrews.

This chapter presents the results of two years of mark-recapture studies on *S.araneus* and *S.minutus* inhabiting a grassland site at Silwood Park near Ascot, Berkshire and removal trapping at two sites (a grassland area and a conifer plantation) in Windsor Great Park.

Since all three sites were within a few miles of each other the parasite communities were unlikely to be show any geographical differences in distribution and since shrews were sampled concurrently at the three sites, the timing of the population changes was likely to be the same in each case. Thus the Silwood population would act as a model of the population changes likely to be taking place in the Windsor populations, but which would be masked by the effect of removal trapping.

Materials and Methods

Study areas were established at three neighbouring locations in Berkshire. One site at Silwood Park (Ordnance Survey Grid Reference SU 945688) near Ascot, was used to monitor population changes of shrews by a mark-release-recapture method. The other two sites at Lipper Pond (SU 950705) and Cranbourne Chase (SU 935736) in Windsor Great Park were used to supply successive samples of shrews for autopsy and examination of their helminth parasites.

In order to supplement the shrews obtained from the two main study areas in Windsor Great Park, some additional trapping was carried out at nearby locations and at Dungeness on the South Kent Coast (TR 074182, Plate 2.2) - table 2.6.

Description of Study Areas

The Silwood site (Plate 2.1) consisted of an area of approximately 1.1 hectares which had been divided into ten plots. Each plot had been ploughed and plants allowed to re-colonise the bare soil, creating a series of plots of different successional ages ranging from two to eleven years old at the beginning of the study (Figure 2.1). Five of these plots, created in 1979, 1980, 1984, 1985 and 1986, were used for the population study of shrews; all five plots were grass-dominated and adjacent to each other.

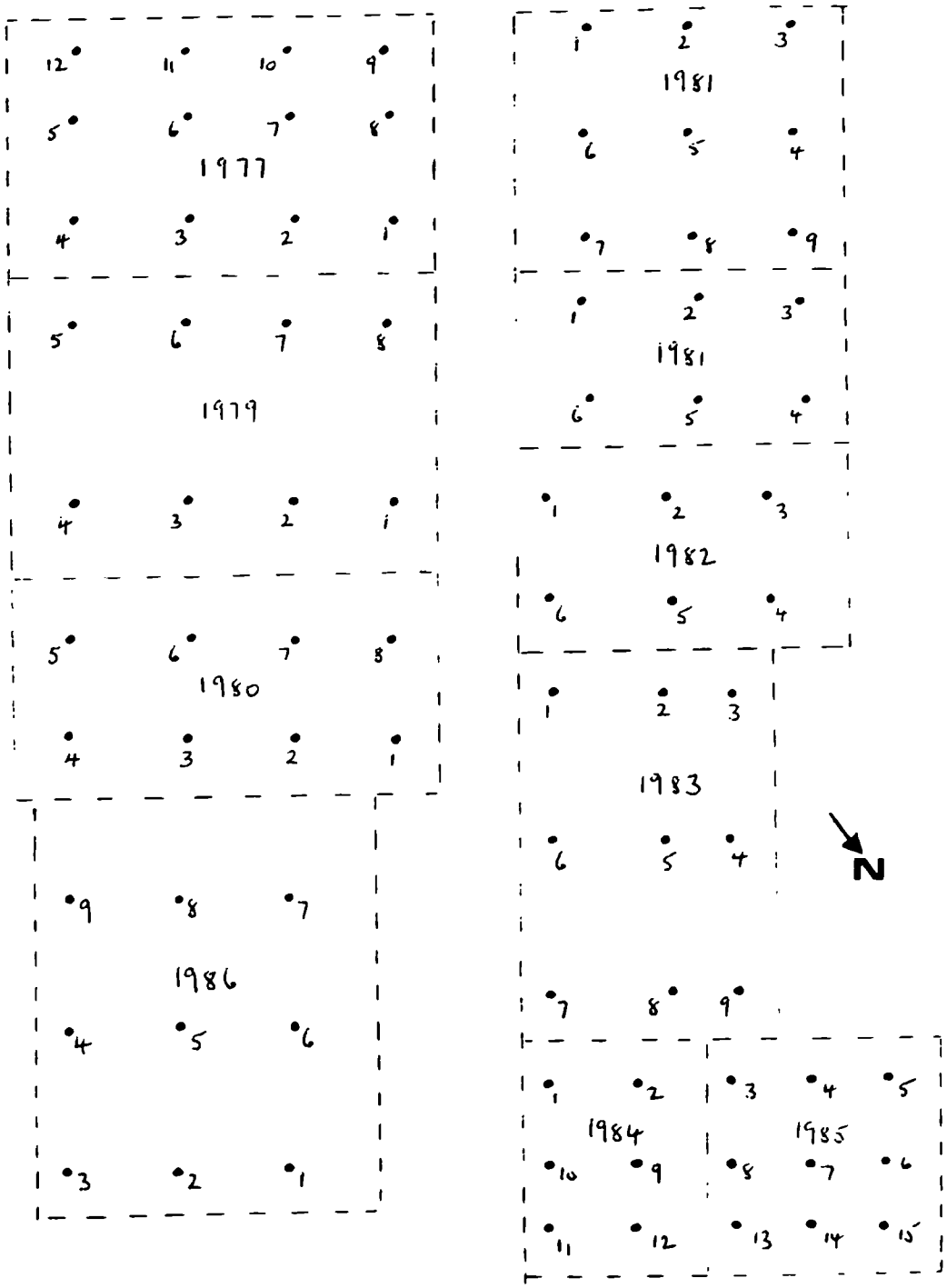
On the 1979 and 1980 plots, approximately 80% of the vegetation cover consisted of the following grasses in decreasing order of abundance: *Holcus lanatus* L., *Agrostis capillaris* L., *Arrhenatherum elatius* (L.) Beauv. ex J. & C. Presl, and *Dactylis glomerata* L. A few oak seedlings (*Quercus robur* L.) and some bramble *Rubus fructicosus* agg. were also present on the 1979 site.

On the 1984 and 1985 plots almost 90% of the cover comprised three grass species: *Elymus repens* (L.) Gould, *Agrostis stolonifera* L. and *Holcus lanatus.*

The vegetation on the 1986 plot was composed mainly of the two grasses, *Elymus repens* and *Holcus lanatus.*

Forb species present on all five plots included *Stellaria graminea* L., *Achillea*

Figure 2.1 - The Silwood successional plots.



• = trap point

——— = 10m

millefolium L., *Cirsium arvense* (L.) Scop., *Rumex crispus* L. and *Senecio jacobaea* L. The soil was a slightly acidic sandy loam.

The site was surrounded on two sides by further grassland plots and on the other two sides by rabbit-grazed grassland with clumps of mature trees. Rabbits were excluded from the study area by means of a wire fence.

The two sites in Windsor Great Park were both managed by the park authorities but in very different ways.

The Lipper Pond site (Plate 2.1) comprised an approximately five hectare area of grassland (mown periodically, but not during the study period) dominated by *Agrostis canina* L., *Holcus lanatus* and *Lolium perenne* L. interspersed with mature trees, mainly oak (*Quercus robur* and a few *Q.petraea* (Mattuschka) Liebl.) with some beech, *Fagus sylvatica* L. and silver birch, *Betula pendula* Roth. Other species present included bracken, *Pteridium aquilinum* (L.) Kuhn, buttercup, *Ranunculus sp.* L. and *Cirsium arvense.*

The soil was a sandy clay loam with a pH of 5.9.

The site was bordered on one side by a road, on another side (separated by a small stream) by short grass which was cut regularly, and on the other two sides by woodland comprising mainly *Quercus robur, Betula pendula, Picea abies* (L.) Karsten (Norway spruce), *Rhododendron ponticum* L., *Pteridium aquilinum* and *Rubus fructicosus.*

The Cranbourne site (Plate 2.2) was a clear fell area covering approximately 7.7 hectares in a section of the park used for commercial forestry. The vegetation consisted mainly of the rush *Juncus effusus* L. and three grasses: *Agrostis canina, Holcus lanatus* and *Festuca gigantea* (L.) Vill. with patches of *Rhododendron ponticum* and *Rubus fructicosus.* Herb species present included *Teucrium scorodonia* L., *Chamaenerion angustifolium* (L.) Scop. and *Senecio sylvaticus* L. Stumps of *Picea abies* which had been felled were present in large numbers as well as some dead branches. *P.abies* seedlings had been planted in rows which were about five metres apart. *Betula pendula* saplings were abundant, a few trees of the following species were also present: Lime (*Tilia europea* L.), ash (*Fraxinus excelsior* L.), chestnut (*Castanea sativa* Miller) and oak (*Quercus robur*). The soil was peaty with a large amount of pine litter and distinctly acidic with a pH of 4.2. The site was


A


B


A


B

surrounded by woodland in which the main plant species were *Picea abies*, *Quercus robur* and *Rhododendron ponticum*.

The differences between the two sites in Windsor Great park allowed an investigation into the effect of habitat on the structures of the two *Sorex* communities and their helminth parasites.

Trapping Methods

Shrews were trapped using Longworth traps (Chitty & Kempson, 1949) containing hay or non-absorbent cotton wool as bedding and baited with fly pupae (*Calliphora sp.*) for shrews, and oats or grain to prevent mortality of any mice or voles captured. Before each sampling period all traps were checked for any damage or malfunction and the treadles were set sensitively enough to be able to catch *S.minutus* weighing as little as 2.8g. Two traps were placed at each trap point. Trapping was carried out under licence from the Nature Conservancy Council (now known as English Nature). Identification, determination of maturity, and sexing of mature shrews was carried out as described by Churchfield (1979).

Silwood Park

Trap points were established at nine metre intervals (Figure 2.1) and marked by means of bamboo canes.

Trapping was carried out at 6-8 weekly intervals during the period October 1988 to September 1990; each trapping session lasted three days and nights.

Between October 1988 and September 1989 the whole site was used making a total of 82 trap points. The traps were left open for the whole of the three day period and checked at approximately 6:30am, 11:30am, 4:30pm and 9pm. Between October 1989 and September 1990 only five of the plots were used: 1979, 1980, 1984, 1985 and 1986, making a total of 40 trap points. The traps were closed at night and re-opened at about 6:30am; they were checked at about 11:30am and just before it got dark.

If a trap was found to be closed it was placed in a large clear plastic bag and

opened so that the animal was immediately transferred to the plastic bag. Shrews were marked by toe-clipping (Churchfield, 1979), under a Home Office Licence, allowing a unique code number to be assigned to each one. The species, code number, state of maturity, sex (if known) and weight (determined using a Pesola spring balance weighing from 0-30g) of each shrew was recorded. The shrew was then released and as many faeces as possible were collected from the tunnel of the Longworth trap and placed in a small plastic specimen tube to be used in the egg-counting procedure (Chapter 4).

Windsor Great Park

Trapping was carried out at two-monthly intervals between November 1988 and September 1990. At the start of each trapping session a grid was marked out using seven rows of seven bamboo canes about fifteen metres apart forming a square approximately 90m by 90m; each cane constituted one trap point. It was sometimes necessary to modify the shape of the grid, but the area covered was kept constant. The location of the grid was moved to a different part of the site at the start of each trapping session to minimise the impact of removal of shrews on the population. Trapping covered a period of three days and nights with the traps being left open for the duration of the period. They were checked two or three times every 24 hours depending on the weather and the abundance of shrews.

Any mice or voles trapped were released while the shrews were placed in large plastic sandwich boxes containing wood chippings, hay and fly pupae, for transport to the laboratory for examination. If a surplus of shrews was obtained the remainder were marked using toe-clipping and released.

Measurement of Soil pH

10g of soil was placed in a beaker with 25ml of distilled water and the resulting suspension stirred for five minutes. The soil particles were allowed to settle and the supernatant was drawn off and pipetted into a 25ml specimen tube.

The pH of the supernatant was measured using a Kent Electronic pH Meter, Model 7060.

Meteorological Data

The temperature and rainfall at Silwood Park has been recorded daily for a number of years by the technical staff of Imperial College, University of London. This data was freely available to the present author.

Collection of Invertebrates

Invertebrates were collected by pitfall trapping between January and November 1990 in order to investigate possible intermediate hosts of shrew parasites. This data could also be used to compare the potential food available at the different study areas. The pitfall trapping method and the invertebrates sampled are described in more detail in Chapter 5.

Results

Population Trends at Silwood Park

Since different trapping routines were used in 1988-9 and 1989-90, only captures made during the day on the 1979, 1980, 1984, 1985 and 1986 plots were used in the calculation of population size.

The absolute population density could not be determined accurately since, as discussed below, calculation of this value requires detailed measurements of the home ranges of the individuals in the population and this was beyond the scope of the present study. It was possible, however, to obtain estimates of population size which could be used to investigate the population cycle in *S.araneus*; numbers of *S.minutus* trapped at Silwood were too low to provide much information on fluctuations in numbers of this species. **In the figures and tables below, numbers of shrews are given as actual numbers trapped on the site concerned unless otherwise stated.**

The size of the population of *S.araneus* was estimated using three different methods (Table 2.1): actual number of individual shrews captured, Hayne's mark-release-recapture method (Hayne, 1949) and an estimate including individuals not captured during the period under consideration, but known to be alive as they were caught at a later date (the calendar of captures method, Petrusewicz & Andrzejewski, 1962). Only individuals caught regularly on the study site were included in the latter estimate in order to eliminate from the calculations non-residents which only occasionally visited the study site, or had emigrated from it. The three estimates give fairly similar values. Table 2.2 shows the numbers from the last estimate converted into numbers per hectare for comparison with other studies. Two sets of values are shown, the first using the actual trapping area and the second using the 'true trapping area' determined with the formula devised by Michielsen (1966):

$$A = L1 \quad L2 + 2L1r + 2L2r + \pi r^2$$

Where A = the effective or 'true trapping area'

L1 & L2 = the length and breadth of the trapping area (assumed to be rectangular)

r = radius of mean home range (assumed to be circular)

This formula was used by Michielsen (1966) to allow for the fact that some of the shrews trapped in a given area had home ranges which extended outside the actual area being studied.

The distribution of the trap points and the number of recaptures did not allow the home range size to be determined accurately, but the cruising radius during the autumn/winter was presumably no larger than 9m (i.e. the shrews were normally confined to an area 18m in diameter) as shrews were rarely trapped at more than one trap point during this period. Results obtained by Churchfield (1984, 1990) suggested that the mean distance moved during a trapping period in the spring/summer (March-September) was double the distance moved in the autumn/winter; a cruising radius of 18m was therefore assumed when calculating the population densities for the spring/summer period. In the present study the maximum recorded distance moved during a sampling period was at least 46m for adults and at least 60m for juveniles.

The minimum estimate of population density of *S.araneus* was 13 shrews per hectare in autumn/winter and 10 in the spring/summer. Maximum population density estimates were 71 individuals per hectare in winter and 79 per hectare in spring/summer.

Figure 2.2 shows the change in population size during the study period (using the results from table 2.1), the numbers peaking in the summer and showing a marked decrease in the autumn, stabilising in the winter and rising again in the following summer. Figure 2.3 shows the changes in number of shrews of each cohort (using the number of shrews known to be alive) indicating the swelling of the population caused by recruitment of juveniles between May and September; juveniles comprised 100% of the population by the autumn. Figure 2.3 shows that a significant proportion of the juveniles as well as the adults appeared to die off in the autumn.

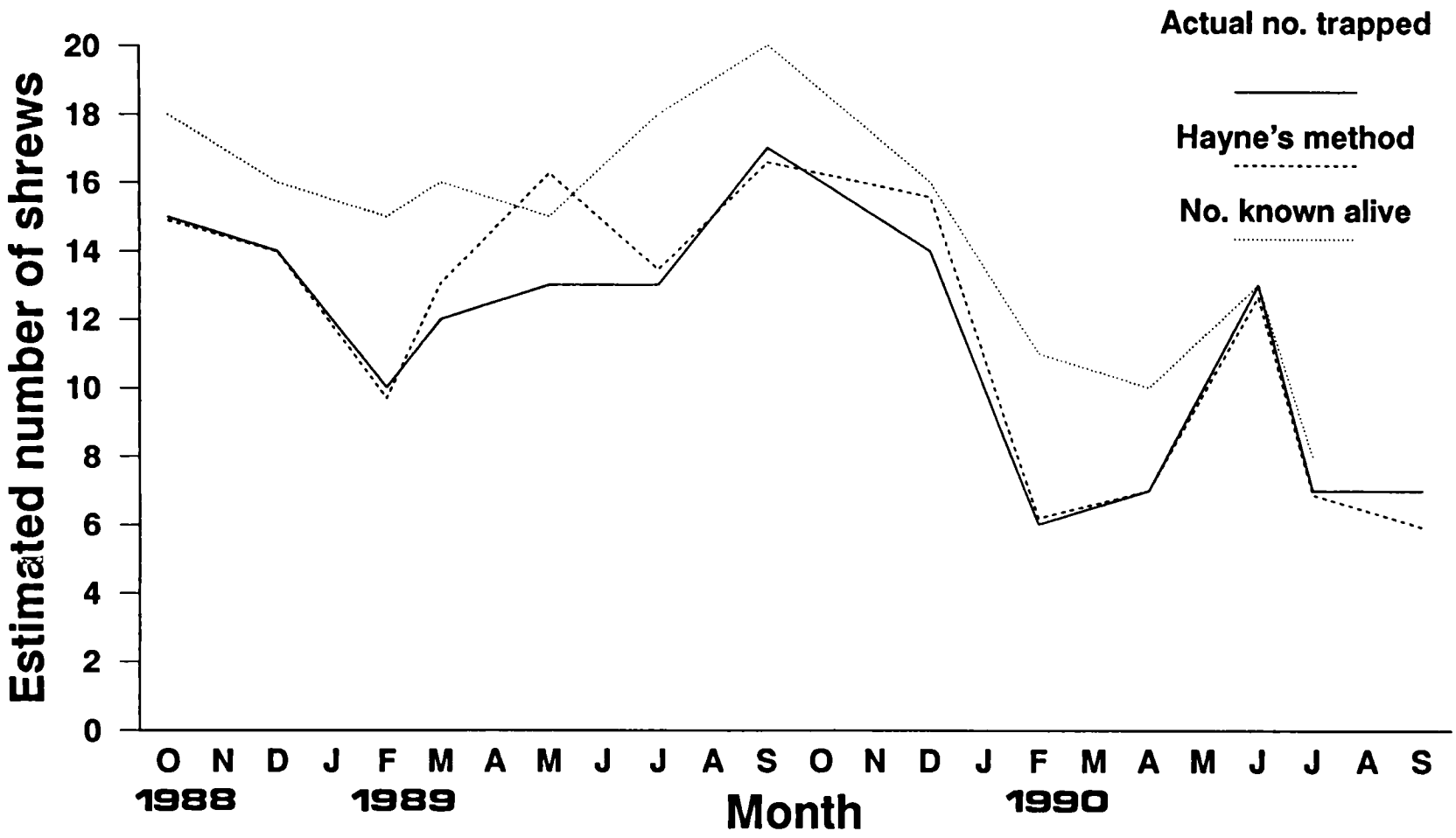
Although the basic trends in population change are similar in the 1988-9 and 1989-90 seasons the size of the 1988-9 cohort is larger than the 1989-90 one and has a longer mean life span.

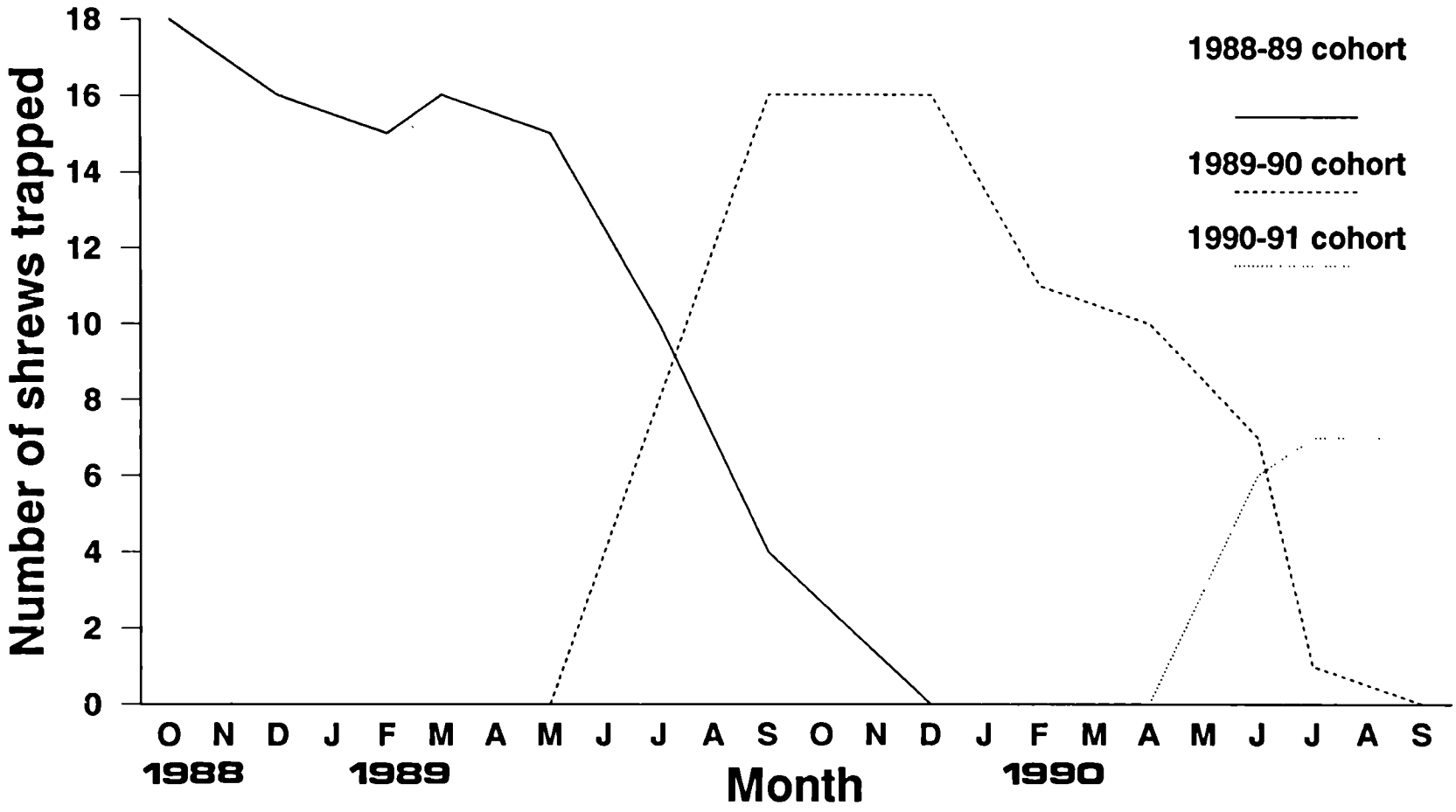
Table 2.1. - Estimates of *S.araneus* population at Silwood.

DATES	POPULATION ESTIMATE No. trapped				Hayne's	No. known alive
	mature ♂♂	mature ♀♀	immature	total		
6-29/10/88	0	0	15	15	15	18
7-10/12/88	0	0	14	14	14	16
8-11/02/89	0	0	10	10	10	15
0-23/03/89	6	6	0	12	13	16
8-11/05/89	10	3	0	13	16	15
0-13/07/89	5	3	5	13	14	18
9-22/09/89	3	1	13	17	17	20
1-13/12/89	0	0	14	14	16	16
9-22/02/90	0	0	6	6	6	11
9-11/04/90	3	4	0	7	7	10
1-13/06/90	2	5	6	13	13	13
3-25/07/90	1	0	6	7	7	8
7-19/09/90	0	0	7	7	6	-

Table 2.2. - Estimates of *S.araneus* numbers per hectare.

DATES	ESTIMATED NUMBER PER HECTARE	
	Actual trapping area	'true trapping area'
26-29/10/88	71	39
07-10/12/88	63	30
08-11/02/89	59	35
20-23/03/89	63	22
08-11/05/89	59	21
10-13/07/89	71	25
19-22/09/89	79	28
11-13/12/89	63	30
19-22/02/90	43	13
09-11/04/90	39	14
11-13/06/90	51	18
23-25/07/90	31	11
17-19/09/90	28	10


Actual no. trapped
Hayne's method
No. known alive
Estimated number of shrews
0
2
4
6
8
10
12
14
16
18
20
O N D J F M A M J J A S O N D J F M A M J J A S
1988
1989
1990
Month


Number of shrews trapped
18
16
14
12
10
8
6
4
2
0
O N D J F M A M J J A S O N D J F M A M J J A S
1988
1989
1990
Month
1988-89 cohort
1989-90 cohort
1990-91 cohort

The population density of *S.minutus* at Silwood (Table 2.3) was much lower than for *S.araneus*. The low numbers trapped did not allow seasonal population changes to be observed.

Table 2.3 - Numbers of *S.minutus* Trapped at Silwood.

DATES	NUMBER TRAPPED mature ♂♂	mature ♀♀	immature	total	NUMBER PER HECTARE (actual trapping area)
26-29/10/88	0	0	0	0	0
07-10/12/88	0	0	0	0	0
08-11/02/88	0	0	2	2	8
20-23/03/89	0	0	0	0	0
08-11/05/89	0	1	0	1	4
10-13/07/89	2	1	0	3	12
19-22/09/89	0	1	1	2	8
11-13/12/89	0	0	5	5	20
19-22/02/90	0	0	2	2	8
09-11/04/90	0	0	0	0	0
11-13/06/90	4	3	0	7	28
23-25/07/90	3	3	0	6	24
17-19/09/90	0	0	0	0	0

Shrew Numbers at Cranbourne and Lipper

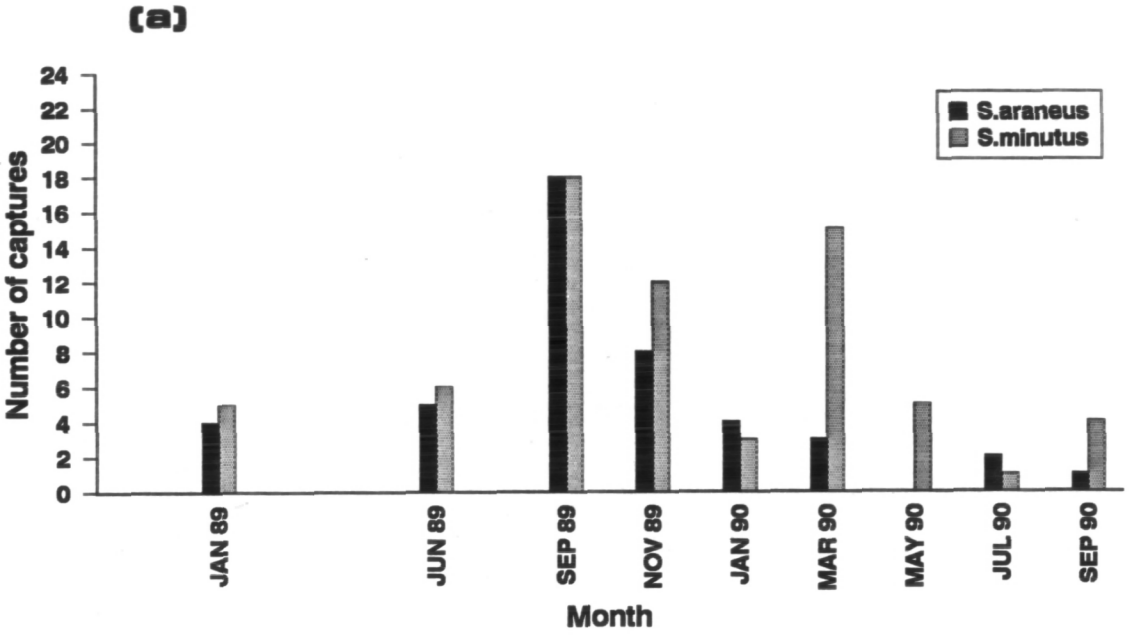
Most of the shrews trapped at the Cranbourne and Lipper sites were removed from the population for laboratory examination although a small number were released as indicated in Tables 2.4 and 2.5. An attempt was made to minimise the effect of removal of shrews from the population by trapping on different parts of the site on successive occasions. However, recapture of individuals marked during a previous trapping period indicated that it was inevitable that the removal of shrews affected the number caught on later trapping expeditions. Numbers of *S.araneus* trapped ranged from 0 to 18 per hectare at Cranbourne (mean=5.0) and from 0 to 24 per hectare at Lipper (mean=5.9). Numbers of *S.minutus* per hectare

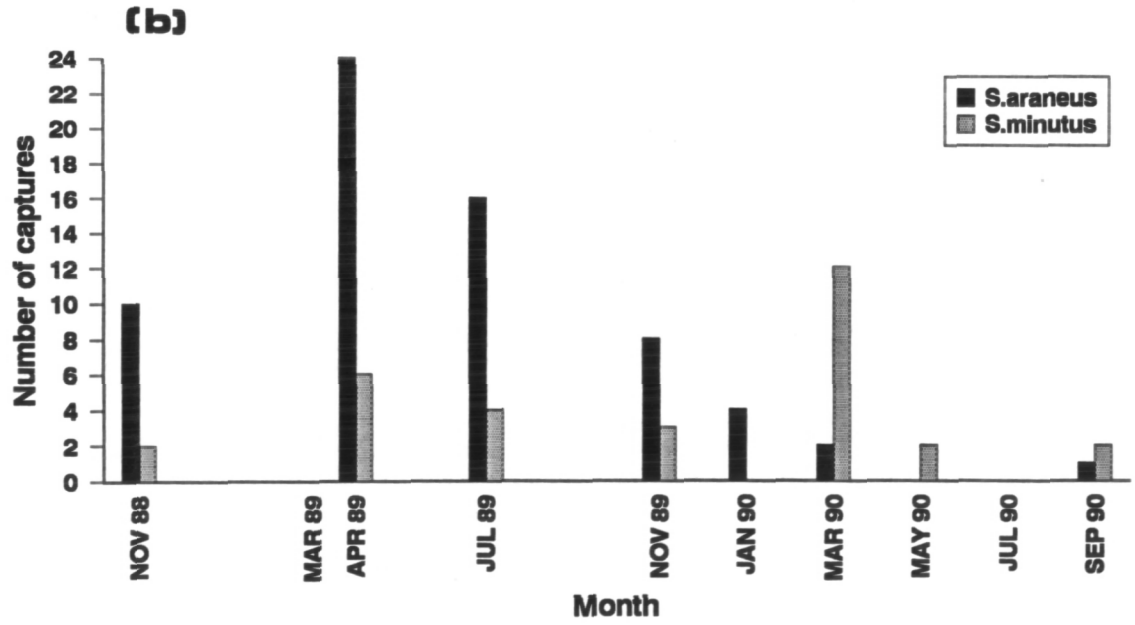
Table 2.4. - Numbers of *S.araneus* and *S.minutus* trapped at Cranbourne Chase, Windsor Great Park.

Dates	No. *S.araneus* trapped (No. kept)	No. *S.minutus* trapped (No. kept)
25-28/01/89	4 (4)	5 (5)
05-08/06/89	5 (5)	6 (6)
12-15/09/89	18 (13)	18 (8)
20-23/11/89	8 (8)	12 (8)
29/1-1/2/90	4 (4)	3 (3)
26-29/03/90	3 (3)	15 (7)
21-24/05/90	0 (0)	5 (5)
17-20/07/90	2 (2)	1 (1)
12-15/09/90	1 (1)	4 (4)
19-22/03/91	5 (5)	2 (2)

Table 2.5. - Numbers of *S.araneus* and *S.minutus* Trapped at Lipper Pond, Windsor Great Park.

Dates	No. *S.araneus* trapped (No. kept)	No. *S.minutus* trapped (No. kept)
21-24/11/88	10 (6)	2 (2)
08-11/03/89	0 (0)	0 (0)
19-22/04/89	24 (24)	6 (6)
17-20/07/89	16 (14)	4 (4)
20-23/11/89	8 (8)	3 (2)
29/1-1/2/90	4 (4)	0 (0)
26-29/03/90	2 (2)	12 (8)
21-24/05/90	0 (0)	2 (2)
17-20/07/90	0 (0)	0 (0)
12-15/09/90	1 (1)	2 (2)
19-22/03/91	0 (0)	0 (0)


(a)
Number of captures
24
22
20
18
16
14
12
10
8
6
4
2
0
S.araneus
S.minutus
JAN 89
JUN 89
SEP 89
NOV 89
JAN 90
MAR 90
MAY 90
JUL 90
SEP 90
Month


(b)
Number of captures
24
22
20
18
16
14
12
10
8
6
4
2
0
S.araneus
S.minutus
NOV 88
MAR 89
APR 89
JUL 89
NOV 89
JAN 90
MAR 90
MAY 90
JUL 90
SEP 90
Month

trapped at Cranbourne ranged from 0 to 18 (mean=7.1) and from 0 to 12 at Lipper (mean=2.8). The difference between captures of shrews at Cranbourne and Lipper was tested using the Mann-Whitney U test; the difference in population density for *S.araneus* was not significant ($P>0.05$) while the population density of *S.minutus* at Cranbourne was significantly greater than at Lipper ($0.01<P<0.025$).

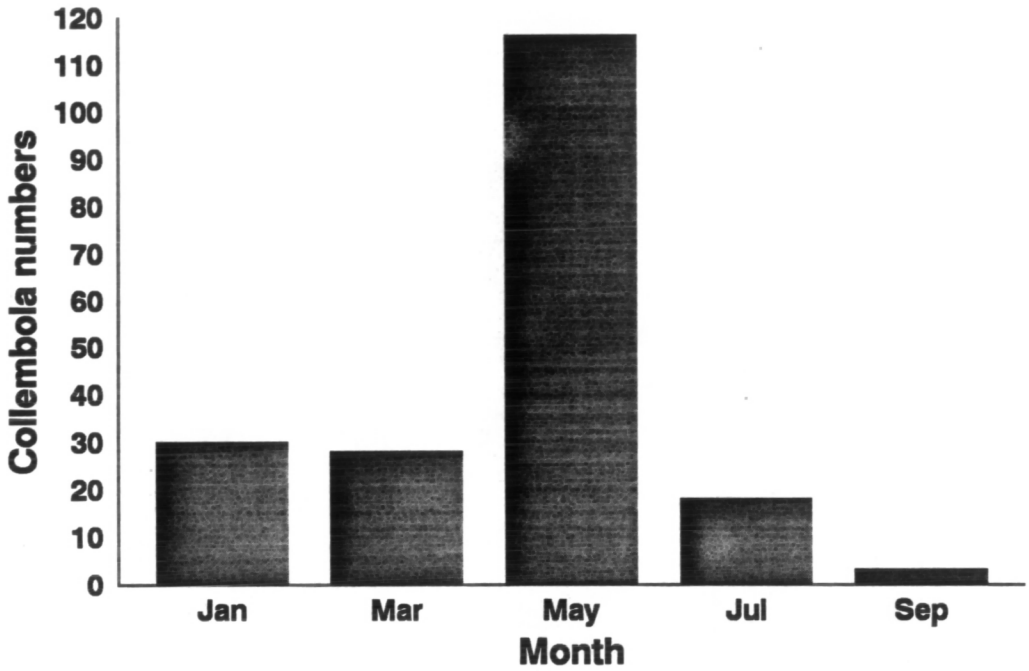
The data on invertebrate numbers pitfall-trapped at the two sites (Chapter 5) were used to construct four bar charts relevant to the present chapter. Figure 2.5 shows the seasonal changes in numbers of Collembola pitfall-trapped at Cranbourne and Lipper. To allow a comparison to be drawn between the two sites and between different times of year, values have been standardised to numbers per twenty pitfall traps per three days (and nights). Collembola were consistently more abundant at Cranbourne than at Lipper.

Figure 2.6 shows the differences in the size composition of the Coleoptera samples from Cranbourne and Lipper. The beetles trapped at Cranbourne were generally smaller than at Lipper since at the former site the most frequent size class was 1-5mm while at the latter site it was 6-10mm.

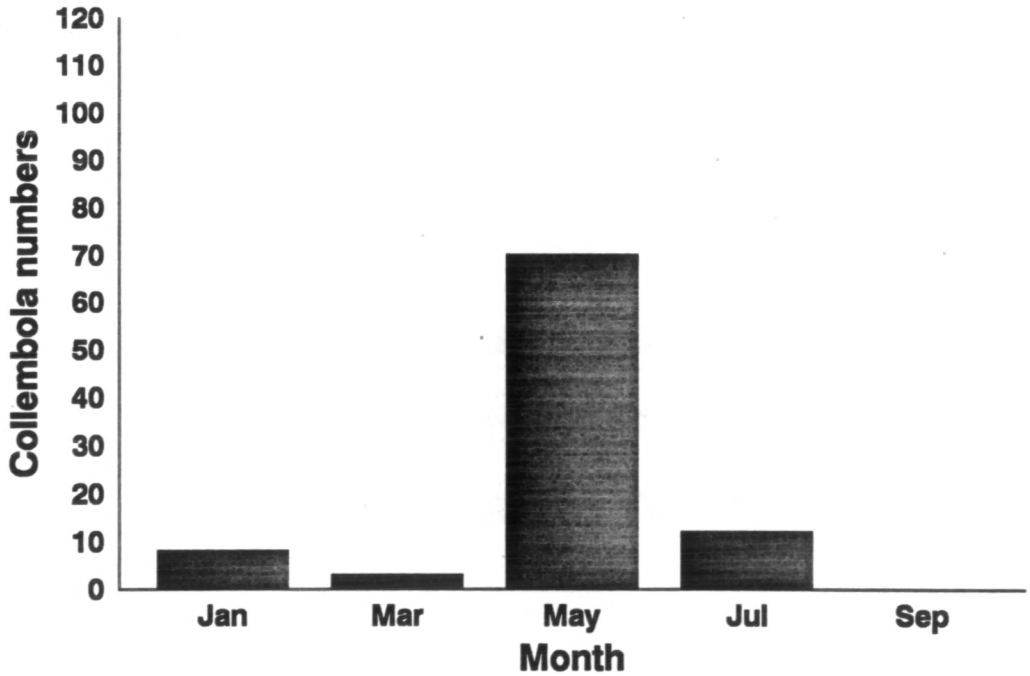
Meteorological Data

The monthly rainfall totals at Silwood Park for the two year period in which trapping was carried out are shown in Figure 2.7. There was much less rain during the summer of 1990 than during the previous summer.

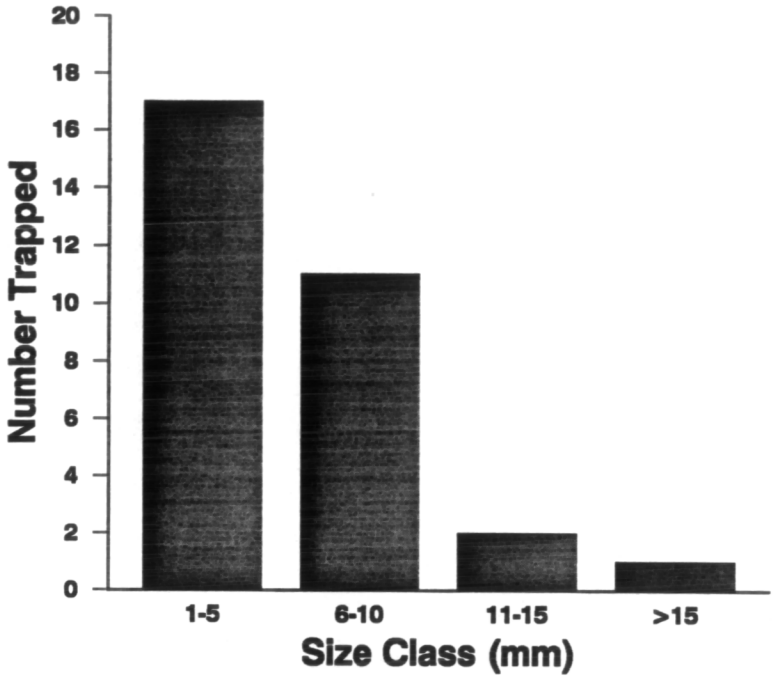
(a)


Collembola numbers
120
110
100
90
80
70
60
50
40
30
20
10
0
Jan
Mar
May
Jul
Sep
Month

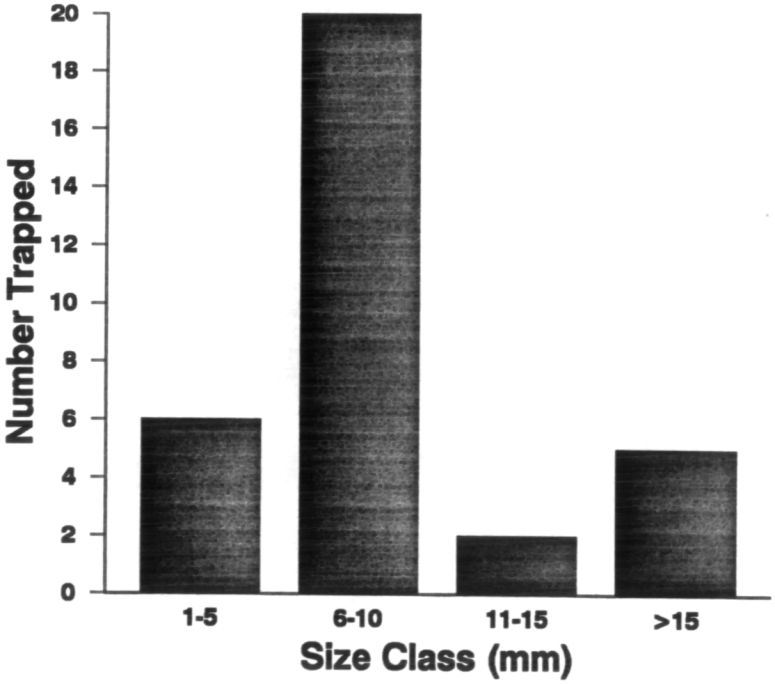
(b)

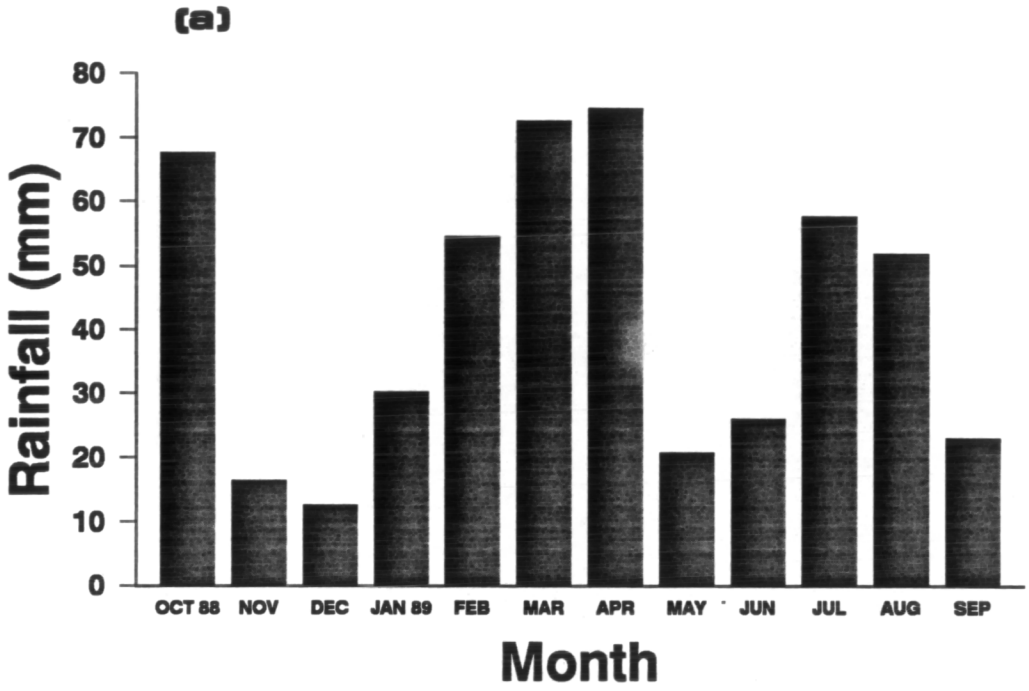

Collembola numbers
120
110
100
90
80
70
60
50
40
30
20
10
0
Jan
Mar
May
Jul
Sep
Month

(a)


Number Trapped
0
2
4
6
8
10
12
14
16
18
20
1-5
6-10
11-15
>15
Size Class (mm)

(b)


Number Trapped
0
2
4
6
8
10
12
14
16
18
20
1-5
6-10
11-15
>15
Size Class (mm)


(a)
Rainfall (mm)
80
70
60
50
40
30
20
10
0
OCT 88
NOV
DEC
JAN 89
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
Month

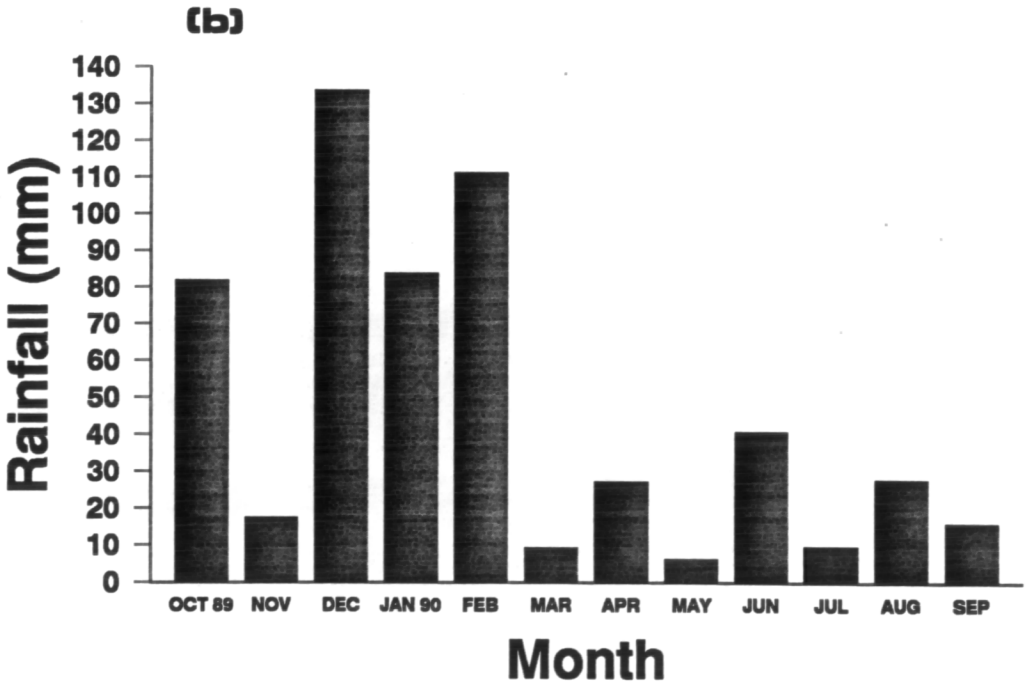

(b)
Rainfall (mm)
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
OCT 89
NOV
DEC
JAN 90
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
Month

Table 2.6. Additional trapping.

Dates	Site	Vegetation	No. of traps	No. *S.araneus*	No. *S.minutus*
27-29/11/87	Upper Norton (SP 310290)	Grass verge	(10 *S.araneus* supplied by Dr S.Mercer)		
24-26/05/90	Alderhurst (SU 997697)	Grass/bramble	50	0	0
11/06/90	Cranbourne (SU 935736)	Rush/grass/bracken	40	0	0
12-15/06/90	Cranbourne (SU 935736)	Rush/grass/bracken	70	0	0
17-20/06/90	Alderhurst bottom (SU 997695)	Rush/grass	80	0	0
21-22/06/90	Silwood (SU 945688)	Rough grassland	42	9	0
23-25/07/90	Silwood (SU 945688)	Rough grassland	80	2	1
17-20/09/90	Silwood (SU 945688)	Rough grassland	80	4	0
25-27/07/91	Dungeness (TR 074182)	Wetland vegetation	100	12	0

Table 2.6. Additional trapping during summer 1990.

Dates	Site	Vegetation	No. of traps	No. *S.araneus*	No. *S.minutus*
24-26/05/90	Alderhurst	Grass/bramble	50	0	0
11/06/90	Cranbourne	Rush/grass/bracken	40	0	0
12-15/06/90	Cranbourne	Rush/grass/bracken	70	0	0
17-20/06/90	Alderhurst	Rush/grass	80	0	0
21-22/06/90	Silwood	Rough grassland	42	9	0
23-25/07/90	Silwood	Rough grassland	80	2	1
17-20/09/90	Silwood	Rough grassland	80	4	0

Discussion

Population Trends at Silwood

The Effect of Changes in Activity of Shrews

The population trends of *S.araneus* over time for Silwood (Figure 2.2) resemble those of Michielsen (1966), Pernetta (1977) and Churchfield (1984). A feature of the above studies as well as the present investigation was an apparent population increase between February and April; this can be observed in both the 1988-9 and 1989-90 cohorts at Silwood as well as in the Lipper Pond data for 1989. A possible explanation for the low numbers found in January and February is that some shrews present in the study area were not trapped during this period.

Churchfield (1984) found that 80% of captures of *S.araneus* were made in spring-summer and only 20% in the winter. Although shrews are known to be more numerous in summer the difference in abundance is not as marked as the above figures suggest and activity must also vary seasonally. Gebczynski (1965) and Buchalczyk (1972) monitored activity of *S.araneus* in the laboratory. Both authors found that activity was lowest in winter and increased abruptly in the spring. Churchfield (1979) also observed captive *S.araneus* and concluded that overwintering shrews were more subterranean, spending less time foraging and more time in the nest to conserve body heat. She also suggested that shrews on the ground surface were less likely to enter traps in the winter. According to Michielsen (1966) *S.araneus* spends 4/5 of its time underground in autumn and winter.

Although shrews are undoubtedly less active in the winter this may not be the only explanation for the low numbers trapped during this period. Some individuals marked during the summer were not recaptured until the following spring. If they had been present in the study site during the winter it is very likely that they would have been trapped at least once during this season. The fact that Hayne's index is approximately equal to the actual numbers captured suggests that all shrews present in the study area were trapped as this method allows shrews present, but not trapped, to be included in the calculation as long as there is an equal probability of trapping each individual. Of course, the

positioning of the traps might make it more likely that certain individuals would be caught rather than others and this effect would be more pronounced if the shrews were less active. It seems likely that some of the shrews emigrated from the study site during the autumn to return in the following spring, but if this is the case an explanation is required as to where they emigrated.

Michielsen (1966) concluded that juvenile *S.araneus* and *S.minutus* established territories in the autumn with no overlap between territories belonging to individuals of the same species. The territories were maintained until the breeding season when territoriality broke down. Buckner (1969) suggested that territories were maintained by the females at least until the first litter had left the nest. Hawes (1977) found a similar state of affairs in *Sorex vagrans* and *S.obscurus* in North America. In the autumn/winter the home ranges were small and did not overlap in shrews of the same species. In the summer the home ranges of the females doubled in size whilst those of the males were at least twice as large again. (There was no difference in home range size between immature males and immature females). There was considerable overlap of home ranges in the spring and summer.

In the present study *S.araneus* were rarely trapped at more than one trap point during the winter suggesting that the cruising radius was less than 9m (trap points were approximately 9m apart) whereas in the summer the maximum distance moved during a sampling period was at least 46m for adults and for juveniles at least 60m. Churchfield (1990) reported that an adult male *S.araneus* was found to travel up to 135m in one day. She found that the mean distance moved by *S.araneus* during a trapping period was 35m between March and September, but only 17m between October and February.

It therefore appears that the apparent increase in population density of shrews in April/May was not due to an increase in the number of shrews resident in the area, but was caused by the nomadic behaviour of mature animals (especially males) looking for mates. The nomadic behaviour of the males at this time of the year is reflected in data obtained by Brambell & Hall (1936): during April and May, 63% of shrews trapped were males whilst during the rest of the year only 49% were males.

Possible Causes of the "Autumnal Epidemic"

The autumnal decline in numbers of *S.araneus* trapped at Silwood Park involved the apparent death of both adult and juvenile shrews (Figure 2.3), the old adults dying off before the juveniles, probably all of them having died by October since no more mature adults were trapped after September. The decline in number of juveniles apparently occurred slightly later than described in previous studies, but nevertheless involved a similarly rapid reduction in numbers.

Various reasons have been put forward for the autumnal decline seen in *Sorex* shrews, some of these applying only to adults, for example senescence, tooth wear and the absence of an autumn moult, others such as inexperience applying only to juveniles, while sociological factors, parasites, predation and depletion of food resources would be expected to affect both adults and juveniles.

Stein (1954) stated that adult shrews did not undergo an autumn moult and therefore did not develop the thick coat necessary for winter survival. However, Pernetta (1976b) showed that at least some adult *S.araneus* have a normal autumn moult; of ten adults trapped in October and November one had the normal summer coat, six were undergoing "patch moults", two a normal autumn moult and one had already grown the winter pelage. As noted by Pernetta (1977) the death of the adults occurs before the onset of the harsh climatic conditions necessitating the growth of the winter pelage. As in the present study he found that most adults disappeared by the end of September.

The possible influence of tooth wear on mortality was first suggested by Spannhof (1952). The effect on a shrew's teeth of chitin in the exoskeletons of arthropods, and soil particles on the surface of earthworms may be serious enough to wear the teeth down to the gums in some cases (Churchfield, 1990). Worn teeth could therefore reduce the efficiency of an adult shrew in handling its prey and thereby increase the likelihood of mortality.

Adult shrews in late summer show several signs of senescence. Adams (1913) reported signs of aging in shrews similar to those found in humans. The hairs of the feet and tail perished from the roots and became gradually shorter until the roots disappeared leaving the skin bald, a similar process to that occurring in

human limbs. The hairs of the ears fell out entirely just like those of the human scalp. Churchfield (1990) found an increasing number of grey hairs in old adults as well as a 'grizzled appearance on the head and rump.' Michielsen (1966) and Churchfield (1990) recorded that shrews in captivity have lived longer than the usual life-span and Michielsen cited three field investigations in which old adults have been found in their second winter. Hence senescence appears to be a contributory factor to the decline of the adults, but is not in itself sufficient to cause death.

Several authors have suggested that parasites might be responsible for the "autumnal epidemic" in shrews, but the evidence is contradictory and inconclusive. In view of the inconclusive nature of earlier research the present study was undertaken in order to carry out a detailed investigation into the role of helminth parasites in the population cycles of shrews. This topic is discussed in Chapter 6.

Southern (1954) reported that *S.araneus* and *S.minutus* were caught more frequently by tawny owls (*Strix aluco sylvatica* Shaw) in the summer and autumn. Casual observations also suggest that shrews are caught more frequently by domestic cats during this period. Crowcroft (1957) noted that the ratio of adult to juvenile shrews in owl pellets during the summer and autumn was very similar to that obtained by trapping during this period; he suggested that predation probably contributed equally to adult and juvenile mortality.

Although predation is apparently greater in the summer and autumn this is probably a reflection of the abundance of shrews rather than an increase in mortality rate of the population.

Crowcroft (1957) suggested that a combination of old age and food shortage was responsible for the autumn mortality. Randolph (1973) devised a model to investigate the energetics of the short-tailed shrew (*Blarina brevicauda*) in Ontario, Canada. He estimated that winter food requirement was 43% higher than the summer requirement due to the lower ambient temperature during the winter period. He also estimated that the population he studied would require only 3% of the total prey base during the summer and about 37% in the winter, the amount of food available on the forest floor being about eight times as much in

summer as in winter.

Although less food is likely to be available in winter Randolph's study suggests that it is not a limiting factor. The decline in the shrew population occurs at a time when food is still freely available.

Some of the factors discussed above, namely senescence, tooth wear and predation appear to contribute to the autumnal decline in numbers of shrews, but are not in themselves sufficient to provide a full explanation. An examination of the territorial and social behaviour of shrews throws more light on the subject.

Aggressive behaviour towards conspecifics is well known in shrews, especially those of *Sorex* species. Churchfield (1990) reported an occasion when two *S.araneus* were so engrossed in a fight that they remained locked in combat even when she picked one of them up by the tail. If two *S.araneus* are caught in the same Longworth trap, a fight will ensue usually resulting in the death of one or both of the shrews. Platt (1976) investigated territoriality in both wild and captive *Blarina brevicauda*. When a shrew was introduced into an occupied enclosure the resident shrew successfully defended the territory, relegating the intruder to an unvegetated area around the perimeter of the enclosure. If the intruder persisted in its attempts to enter the occupied territory it was usually killed by the resident shrew. Churchfield (1990) observed similar behaviour in captive *S.araneus*.

Moraleva (1989) suggests that breeding female *S.araneus* are dominant to juveniles which are themselves dominant to old males. Juvenile shrews reared in captivity have been known to attack and kill their mother after weaning (S. Mercer, personal communication). Michielsen (1966) concluded that juveniles were socially dominant to the adults.

The existence of territorial behaviour in *Sorex* species has already been described above. It appears that juveniles set up territories in late summer and are able to defend them against the socially subordinate adults. Juveniles unable to find an occupied territory and adults which have been ousted from theirs will be threatened or attacked by resident animals as they cross their home ranges; encounters between conspecifics will be frequent as population densities are high. A combination of stress from such encounters, old age, the stress of breeding and increased vulnerability to predation will increase the chances of mortality in old

adults. Juveniles will also suffer from stresses associated with territorial conflicts and vulnerability to predation. If relegated to marginal habitats, inability to find sufficient food may also increase mortality.

It appears that intraspecific conflict has an important influence on mortality. Shrews are reluctant to enter an occupied territory and those which do are usually expelled by vocalisations rather than actual fighting. Hence a resident shrew will suffer less conflict than a nomadic one; it is therefore advantageous for an individual to establish a territory.

The avoidance of potentially harmful encounters with conspecifics is not the only possible function of territorial behaviour. Holling (1959) found that densities of *Sorex cinereus* populations were related to prey densities. Possession of a territory will ensure sufficient food is available during the winter when prey may be less abundant.

In the light of the above evidence a likely explanation for the observed population cycles is as follows. Juvenile *S.araneus* have established territories by late summer, some travelling over 100m in order to do this (Churchfield, 1990). Juveniles unable to defend territories and (socially subordinate) adults die off in the autumn. Most shrews survive the winter, a small percentage being killed by predators and other factors. Numbers of shrews caught in Longworth traps may be lower than expected in January and February due to a decrease in activity on the ground surface. During the spring the population seems artificially high as mature individuals, especially males, range over large distances in search of mates. As the summer proceeds the number of captures increases dramatically as large numbers of juveniles are recruited into the population, the apparent population density perhaps being a little larger than the true value due to wandering juveniles seeking to establish a territory.

The Effect of Climate on Population Size

A clear difference in the number of shrews overwintering can be seen when comparing the 1988-9 and 1989-90 cohorts of *S.araneus* at Silwood Park. The lower number of shrews overwintering in 1989-90 may be one of the reasons for the

small number of shrews recruited into the 1990-91 cohort. Another reason for this might be the very hot dry weather from April to September 1990. The rainfall during this period was much less than in the corresponding period of 1989 (Figure 2.7). It was extremely difficult to obtain shrews from any site during the summer of 1990 as can be seen from Tables 2.4, 2.5 and 2.6. A similar difficulty in trapping small mammals of any species was also encountered by Twigg (personal communication) in the summer of 1976. The summers of both 1976 and 1990 were very hot and dry so drought may have an adverse affect on shrew numbers. Churchfield (1990) suggested that drought might cause a decrease in the numbers of invertebrates available and so cause a decline in the shrew population.

Borowski & Dehnel (1952) and Mystkowska & Sidorowicz (1961) showed that shrews were more likely to trapped during rainy weather so the smaller numbers trapped during the summer of 1990 may reflect a difficulty in trapping shrews rather than a decrease in their abundance.

Aulak (1970), Mezhzherin (1960) and Yalden (1974) recorded distinct variations in abundance of shrews from year to year, but do not give a satisfactory explanation for this. Michielsen (1966) found that in *S.araneus* and *S.minutus* each generation reached a certain level of numbers in the autumn which did not vary much from year to year except for the 1958-9 population of *S.minutus* which was much smaller than usual. She attributed this to the low proportion of the females which survived the previous winter.

Hence it appears that climatic factors may be an important influence on fluctuations in the size of shrew populations and their trappability.

Population Densities of Shrews

The problems involved in the estimation of the population density of shrews have already been considered above. A conservative estimate of the population density of *S.araneus* at Silwood park would be at least 30 per hectare, significantly larger than even the maximum estimates for the Windsor Great Park sites. This may be due to a greater abundance of prey, but this is difficult to substantiate from the limited sampling of invertebrates which was carried out (Chapter 5). A

figure of 30 individuals per hectare is within the range reported in other studies (Churchfield, 1990).

Comparison of the *Sorex* Communities at Cranbourne Chase and Lipper Pond

A comparison of the shrew populations at Cranbourne and Lipper suggests that *S.araneus* was slightly more abundant at Lipper than at Cranbourne, but the difference was not statistically significant. However the difference in population size of *S.minutus* at the two sites was considerable, the value for Cranbourne being about 2.5 times that for Lipper. *S.minutus* outnumbered *S.araneus* at Cranbourne. These observations can be linked to the peaty nature of the soil at Cranbourne.

Yalden (1981) and Butterfield et al. (1981) showed that *S.minutus* was more numerous than *S.araneus* on peaty moorland sites, sometimes outnumbering it by 30:1. Both authors attributed this to the nature of the food available. Earthworms, which form a large proportion of the diet of *S.araneus*, are not common in peaty soils (Edwards & Lofty, 1977). Yalden (1981) measured the sizes of invertebrates caught in pitfall traps on moorland and suggested that they were more suitable to *S.minutus* than *S.araneus*.

However, Dickman (1988) suggested that the size of prey taken by *S.minutus* was influenced by competition with *S.araneus* rather than feeding preference, *S.araneus* tending to exclude its smaller counterpart from habitats in which larger prey could be found thus obliging *S.minutus* to forage in habitats where the mean prey size was smaller.

The larger ratio of *S.minutus* to *S.araneus* on peaty sites cannot merely be attributed to *S.minutus* competing more successfully against *S.araneus* because Grainger & Fairley (1978) found that in Ireland where *S.araneus* is absent the highest population densities of *S.minutus* were in conifer plantations (where the soil is peaty) despite there being less cover than at some of the other sites investigated.

A comparison of the *Sorex* community structures at Cranbourne and Lipper also suggests that the abundance of *S.minutus* is not adversely affected by the

presence of *S.araneus* since the size of the *S.araneus* population is very similar at both sites.

Since invertebrates had been sampled at both sites (in order to look for parasite larvae), the invertebrate fauna at Cranbourne and Lipper could be compared. The major difference between the two sites was the huge numbers of Collembola found at Cranbourne (Figure 2.5). Although *S.minutus* are known to eat Collembola there is no evidence in the literature that they form a significant proportion of the diet, they may, however, provide food for small predatory beetles on which *S.minutus* feeds. There is a difference in the size composition of the Coleoptera samples obtained at each site (Figure 2.6). Pernetta (1976a) found that *S.minutus* ate adult Coleoptera in the 2-6mm range and Dickman (1988) found that over 50% of the food of *S.minutus* was 1-5mm in length and over 80% was less than 10mm in length. The results obtained by Churchfield (1990) are slightly different - with prey in the 6-10mm range apparently being preferred to those in those 5mm or less, but nevertheless show that over 80% of prey is 10mm or less. The Coleoptera available at Cranbourne therefore appear to be more suitable to *S.minutus* than those found at Lipper. Thus food availability may be important in determining the greater abundance of *S.minutus* on peat sites, but further work is required to substantiate this.

Summary

(1) The population cycles of high numbers in summer, a decline in autumn and low overwintering numbers, seen in *S.araneus* in the present study, were similar to those recorded by previous authors; the sample sizes were too small to elucidate population trends in *S.minutus*.

(2) The Silwood population cycles provided a model for the population cycles taking place at Lipper and Cranbourne.

(3) The autumnal die-off of adult shrews appears to be due to old age, exacerbated by sociological factors, but other factors such as parasitism may also contribute to mortality.

(4) A significant proportion of the juveniles also die-off and it is suggested that

sociological factors associated with territoriality may play a major part in this. The possible role of parasites in influencing population cycles of shrews is discussed in Chapter 6.

(5) *S.minutus* is more common at the peaty Cranbourne site and this may be due to the structure of the invertebrate community, more small arthropods suitable for *S.minutus* being found at this site.

Chapter 3
Morphology and Taxonomy of Helminth Parasites from *Sorex araneus* and *S.minutus* in Britain.

Introduction

The first records of helminths from British shrews were those of Baylis (1928, 1934, 1939), Morgan (1928), Baylis & King (1932) and Cameron & Parnell (1933). During this period, knowledge of the occurrence of helminths in Britain was very limited so that parasitological research often took the form of general surveys of helminths present in whatever hosts were available to the authors at the time.

During the 1950s and 60s the spectrum of hosts examined in an individual investigation was narrowed and research on British shrew parasites recorded from this period was generally carried out as part of a survey of the helminth parasites of rodents and insectivores from selected sites. These surveys were not satisfactory for the purpose of building up an accurate and comprehensive picture of the helminth fauna in British shrews. James (1954) examined only 19 specimens of *Sorex araneus* and eight of *S.minutus*. Thomas (1953) examined a larger sample of 72 specimens of *S.araneus*, but did not examine *S.minutus* and concentrated purely on nematodes and digeneans.

None of the above helminthological investigations or any of the others carried out before the present study, involved a thorough investigation of all helminths present in the shrews: generally only the alimentary canal and (sometimes) the body cavity was examined. Lewis (1964, 1968) appears to be the only author to have examined the urinary bladder, gall bladder and bile duct. The lungs and kidneys of British shrews do not appear to have been examined although European workers have shown them to harbour helminth parasites. In addition to this rather incomplete nature of previous studies on the helminth parasites of *Sorex* species in Britain, another problem appears to be incorrect identification of the parasites found. This can be attributed to inaccuracies in reference material used by the authors and incomplete examination of the parasites recovered as is explained below.

Thus a detailed investigation of the helminth faunas of British *S.araneus* and *S.minutus* would be an invaluable contribution to the existing literature on this subject.

Several large-scale surveys of the helminth parasites of *S.araneus* and *S.minutus*

have been carried out in other European countries among them work by Soltys (1952, 1954), Prokopic (1959) and Mas Coma & Gallego (1975) and the taxonomy of some of the groups of helminths found in shrews has been reviewed (Drozdz 1970, Vaucher 1971, Moravec, 1982). However there is a need both for a review of the taxonomy of certain species, particularly nematodes, and for a re-examination of the literature in the light of current knowledge.

A thorough understanding of the parasites present in British *Sorex* species, particularly those in the Windsor and Silwood sites was necessary in order to investigate accurately the effects of the parasites on their host population cycles. A comprehensive knowledge of the morphology of the parasites was required both to identify correctly parasite species present in shrews, and to identify the larval stages of parasites present in invertebrate intermediate hosts. A knowledge of the characteristics of the eggs of the helminth parasites investigated was necessary in order to identify helminth eggs present in samples of shrew faeces.

The present chapter considers the results of a comprehensive investigation of the helminth parasites found in British specimens of *S.araneus* and *S.minutus* with particular reference to those obtained from Windsor Great Park and Silwood Park, Berkshire. Also included in this chapter are up-to-date lists of the species of helminth parasites recorded from *S.araneus* and *S.minutus* in Europe.

Materials and Methods

Maintenance of Shrews

Shrews were obtained using the procedures described in Chapter 2. In order to obtain parasites in optimum condition shrews were examined, where possible, immediately after culling. The maintenance procedure described below was developed in order to keep the shrews alive until required. The shrews were kept in an animal house room with natural lighting, in plastic bins 60cm long, 35cm wide and 30cm deep, or in aquarium tanks of similar dimensions. The floor of the bin was covered with sawdust and hay to a depth of a few centimetres. A small glass pot containing water and another one containing maggots or pupae as food were provided.

Shrews could be kept alive for several weeks under these conditions, but they were generally culled, using ether or chloroform vapour, after two or three days to eliminate any significant change in their helminth fauna.

Shrews found dead in the traps which could not be examined immediately were preserved by freezing or in some cases by placing them in 5% formol-saline.

Examination of Shrews

The fur of the shrew was carefully searched under a low power binocular microscope for any ectoparasites, these were fixed in 70% alcohol. The weight, tail length, and length of head and body were then recorded. The shrews were skinned and any parasites located in the body cavity were removed, identified and fixed. The sex of the shrew was now confirmed by examination of the genitalia.

The shrew was dissected and each organ removed and examined in physiological saline. The bladder, stomach and oesophagus were cut open with fine scissors whereas the liver, gall bladder and bile duct, kidneys, and lungs were each examined by carefully squashing them between two slides. This was done very carefully so that any parasites found could be removed undamaged. The intestine was measured and cut into three roughly equal sections attempting,

where possible, not to cut any cestodes present. Each section was placed in a petri dish of physiological saline and cut open longitudinally. The numbers and species of each parasite present in each organ were recorded on a record sheet.

The spleen and pancreas of Ascelli (a large ovoid lymph gland located in the mesentry just ventral to the anterior pole of the kidneys) were removed and weighed following the removal of excess fluid with absorbent paper.

Fixation and Examination of Parasites

Using fine needles or a pipette, parasites were transferred to a watch glass of saline and examined when fresh, most taxonomically important features being visible without staining or clearing being required. If necessary the parasites were fixed and stained as described below.

Nematodes

For light microscopy worms were relaxed by placing them in a watch glass of saline on top of a hot plate or oven for about 15 minutes. Most of the saline was then removed using a pipette and replaced by 70% alcohol at a temperature of 60-70^{0}C. The worms were stored in 70% alcohol.

Nematodes were first examined in water. If the diagnostic features such as oesophagus, excretory pore and spicules could not be seen clearly a drop of lactophenol was placed on the slide on one side of the coverslip and water was drawn off from the other side of the coverslip using absorbent paper. This process was stopped as soon as the relevant features could be seen, otherwise the worms tended to become almost completely transparent. After examination the coverslip was soaked off in a petri dish of water and the worms transferred through an alcohol series to 70% alcohol.

For scanning electron microscopy (SEM), nematodes were fixed in a solution of 3% glutaraldehyde in 0.1M phosphate buffer for a minimum of 1.5 hours and then placed for fifteen minutes in each of the following solutions: two rinses of 0.1M phosphate buffer, 20% alcohol, 35% alcohol, 95% alcohol and two rinses of absolute alcohol. The specimens were critical point dried in a Biorad Critical Point

Dryer and sputter coated in a Polaron SEM Coating Unit at a current of 20mA for 2-3 minutes. They were examined using a Cambridge Stereoscan 100 electron microscope.

The synlophe was examined in transverse section following the procedure described in Durette-Desset (1982). Sections were stained with astra blue to make them more visible.

Digeneans

Digeneans were relaxed by placing them in warm saline, fixed for one minute in Berland's fluid (19 parts glacial acetic acid: 1 part pure formalin) and stored in 70% alcohol before staining in Mayer's paracarmine (see below).

When placed in Berland's fluid the digeneans tended to contract before the fixative had time to work. This could be prevented by placing them between two microscope slides and lowering the pair of slides into a petri dish of Berland's fluid.

Cestodes

Cestodes were flattened by placing them on a microscope slide with a small dot of petroleum jelly at each end and placing another slide on top. Sometimes it was necessary to compress the slides together using an elastic band. The pair of slides was placed overnight in a petri dish containing 5% formol-saline to fix the cestodes in a flattened state, allowing the internal structures to be seen more clearly. After flattening the slides were teased apart and the specimens transferred through an alcohol series to 70% alcohol before staining in Mayer's paracarmine.

In order to accurately measure and count the hooks on the rostellum of a cestode, it was sometimes necessary to adopt the following procedure: a fresh specimen was placed in a small amount of saline on a microscope slide. The tip of the scolex was then sliced off using a small scalpel blade. The small portion obtained was transferred to a drop of saline or water on another slide using a brush with three bristles. The section was then manoeuvred by means of the brush so that an 'en face' or 'head on' view of the rostellum could be obtained. A coverslip was then lowered carefully onto the section and the slide examined

under a microscope.

The rostellar hooks could be seen more easily by separating them from the rostellum; this could be achieved by pressing on top of the coverslip with a mounted needle.

Acanthocephalans

The acanthella stages were excysted by placing them overnight in distilled water in the refrigerator. The water absorbed by the acanthella caused the proboscis and tail to be everted. The specimens were either placed in 70% alcohol for storage or prepared for SEM as previously described for nematodes.

Staining with Mayer's Paracarmine

The stain was made by dissolving 1g of carminic acid, 0.5g of aluminium chloride and 4g of calcium chloride in 100ml of 70% alcohol. For ease of transfer between the various solutions used in the staining process, parasites were pipetted into small staining baskets which consisted of a short piece of plastic tubing 1.2cm in diameter and approximately 2cm in length with a base made of nylon mesh. Baskets with different mesh sizes were used depending on the parasite's dimensions. The baskets could easily be transferred by means of forceps between the various solutions which were contained in small glass vials 2.2cm in diameter and 3.3cm in height.

The following solutions and immersion times were used:

(1) Mayer's Paracarmine: 10-20 minutes depending on the specimen.
(2) Acid alcohol (1:99, concentrated HCl:70% alcohol): 1 minute
(3) 70% alcohol: 10 minutes
(4) 90% alcohol: 10 minutes
(5) Absolute alcohol: 10 minutes
(6) A second vial of absolute alcohol: 10 minutes
(7) Xylene: 10 minutes

The parasites were transferred from the xylene onto a microscope slide using a brush with three bristles. A couple of drops of Canada balsam, DPX or XAM

were placed on top of the specimen and a coverslip gently lowered on top of it using a mounted needle.

Measurements of the various structures of the parasites were made either using an eyepiece graticule or from drawings made with the aid of a camera lucida.

List of Parasite Species Identified

A total of 129 *Sorex araneus* and 75 *S.minutus* were examined from the following locations: Cranbourne Chase, Lipper Pond, Silwood Park, Dungenesss, "Alderhurst" and "Huntersdale" (Department of Biology, RHBNC), Upper Norton, Oxfordshire, and Chesham, Buckinghamshire (Chapter 6). The helminth fauna of *S.araneus* comprised 10 species of cestodes, 13 species of nematodes, 3 species of digeneans and 2 of acanthocephalans. In *S.minutus*, 8 species of cestodes, 13 nematodes, 2 digeneans and 1 acanthocephalan were recorded. These helminths are listed in Table 3.1 along with their location in the host. Several of the helminths identified were new records for Britain namely the cestodes, *Hymenolepis infirma*, *H.jacutensis*, *H.prolifer* and *H.schaldybini*, the nematode *Stefanskostrongylus soricis*, and the digenean, *Opisthioglyphe sobolevi*. *Sorex minutus* was recorded as a new host for *E.kutori*.

Ectoparasites were not studied in detail, but the following species were identified: larval mites in the fur of *S.araneus*; the tick, *Ixodes (Exopalpiger) triangulicep*s Birula, 1895 in both shrew species; the fleas, *Doratopsylla dasycnema* (Rothschild, 1897) in *S.minutus* and *Hystrichopsylla talpae talpae* (Curtis, 1826) in *S.araneus*.

Morphology of the Helminth Parasites

The majority of the parasites found have already been described satisfactorily. However, most of the species have not previously been described from Britain hence details of measurements of taxonomically relevant structures have provided a useful addition to the literature. In a few cases the descriptions of the parasites are incomplete, for example *Parastrongyloides winchesi* has not been described since the original description by Morgan (1928) and in such cases a more detailed description has been given. SEM studies on the acanthocephalan *Gordiorhynchus aluconis* and the nematode *Stefanskostrongylus soricis* are also described for the first time. **In the Tables 3.2 to 3.30 all measurements are given in μm unless otherwise stated.**

Table 3.1. List of helminth species found in *S.araneus* and *S.minutus* and their location in the host.

Helminth	Present/absent *S.araneus*	Present/absent *S.minutus*	Location	
Cestodes:				
Choanotaenia crassiscolex	✓	✓	Small intestine	
Choanotaenia hepatica	✓	x	Bile duct	
Hymenolepis diaphana	✓	✓	Intestine	
Hymenolepis furcata	✓	✓	Intestine	
Hymenolepis infirma	✓	✓	Intestine	*
Hymenolepis jacutensis	✓	✓	Intestine	*
Hymenolepis prolifer	✓	x	Intestine	*
Hymenolepis schaldybini	✓	✓	Intestine	*
Hymenolepis scutigera	✓	✓	Intestine	
Hymenolepis singularis	✓	✓	Intestine	
Digeneans:				
Brachylaemus fulvus	✓	✓	Oesophagus, stomach	
Dicrocoelium soricis	✓	✓	Gall bladder, bile duct	
Opisthioglyphe sobolevi	✓	x	Intestine	*

* = New British Record # = New Host Record

Table 3.1. (Contd) Helminth species found in *S.araneus* and *S.minutus* and their location in the host.

Helminth	Present/absent			Location	
	S.araneus	*S.minutus*			
Nematodes:					
Calodium cholidicola	✓	x		Liver	
Eucoleus oesopagicola	✓	✓		Oesophagus	
Eucoleus kutori	✓	✓	#	Stomach	*
Liniscus incrassatus	✓	✓		Bladder	
Parastrongyloides winchesi	✓	✓		Intestine	
Porrocaecum sp. (larva)	✓	✓		Body cavity, liver, gut wall	
Nematoda sp. a (larva)	✓	x		Liver	*
Nematoda sp. b (larva)	✓	✓		Liver, oesophagus & gut wall	
Stammerinema soricis	✓	✓		Stomach	
Stefanskostrongylus soricis		✓		Lungs	*
Longistriata depressa	✓	✓		Intestine	
Longistriata didas	✓	✓		Intestine	
Longistriata thomasi	✓	✓		Intestine	
Longistriata trus	✓	✓		Intestine	
Acanthocephala:					
Gordiorhynchus aluconis	✓	✓		Body cavity	
Prosorhynchus sp.	✓	x		Intestine	

Cestodes

Family Dilepididae Fuhrman, 1907.

Choanotaenia crassiscolex, found in the small intestine of *S.araneus* and occasionally *S.minutus,* is recognized by its large scolex with a double row of characteristic hooks. The suckers were larger than recorded by Vaucher (1971) and the number of hooks was less variable (Table 3.2). The eggs were larger than recorded by Vaucher (1971); the dimensions of the embryophore (34-41 x 20-25μm) are recorded for the first time.

Table 3.2. Morphometric data on *Choanotaenia crassiscolex* (present study).

Characteristic	No. examined	Range	mean	S.D.	After Vaucher (1971)
Length/mm	2	-	9.0	-	18
Max width[1]/mm	1	-	1.6	-	1.3
Scolex width	1	-	646	-	375-506
Rostellum length	1	-	498	-	343-400
Sucker length	2	580-660	620	-	242-466
Sucker width	2	264-312	288	-	106-210
No. of hooks	-	18-20	-	-	17-24
Hook length	2	44-50	47	-	35-48
Egg length	8	46-54	50	3.1	38-50
Egg width	8	27-31	28	1.3	20-27
emb.[2] length	9	34-41	38	2.4	-
emb. width	9	20-25	23	1.5	-

(All measurements in μm unless otherwise stated.)

[1] width of strobila at its widest part

[2] emb. = embryophore

C.hepatica is found in the bile duct of *S.araneus*. Specimens are up to 2cm long, with a large scolex which possesses round suckers and a mushroom-shaped rostellum armed with 46 hooks (Plate 3.1, Figure 3.1). The rostellar hooks are arranged in two irregular rows. The double row of hooks and large number of testes (38-48) is diagnostic of the family Dilepididae. Morphometric data on *C.hepatica* is shown in Table 3.3. A comparison of the present data on *C.hepatica* with that of previous authors (Table 3.4) shows that the specimens described in the present study are similar to those previously described as *C.hepatica* although the scolex and eggs are larger than recorded previously.

Table 3.3 Morphometric data on *Choanotaenia hepatica* (present Study).

Characteristic	No. examined	Range	mean	S.D.
Length/cm	2	8-10	9.0	-
Max width/mm	3	0.211-13.200	4.560	7.482
Scolex length	4	598-898	808	148.0
Scolex width	4	521-618	580	45.6
Rostellum length[1]	3	188-228	209	20.2
Sucker length	16	144-191	170	13.3
Sucker width	15	117-201	142	20.0
No. of hooks	4	46-46	46	-
Hook length	10	34-40	38	2.1
No. of testes	13	38-48	42	2.7
Egg length	9	44-45	44	0.7
Egg width	9	34-37	34	1.2
Oncosphere hook length	2	-	11	-

(All measurements in μm unless otherwise stated.)

[1] includes length of rostellar sac

Table 3.4. Measurements of *Choanotaenia hepatica* compared with previous authors.

Characteristic	Baer (1932)	Zarnowski (1955)	Vaucher (1971)	Present study
Length/cm	1.5	9.5-10.5	3.2	8-10
Max width/mm	0.500	1.650-1.700	-	0.211-13.200
Scolex length	-	-	183-274	598-898
Scolex width	460	400-420	260-353	521-618
Rostellum length	-	192	86-114	188-228[1]
Sucker length	130	150	86-114	144-191
Sucker width	130	150	93-187	117-201
No. of hooks	46	48	c.45	46
Hook length	38	39	35-37	34-40
No. of testes	-	45-50	50-53	38-48
Egg length	-	33-37	26-32	44-45
Egg width	-	33-37	24-30	34-37
Oncosphere hook length	-	-	9-10	11

(All measurements in μm unless otherwise stated.)

Discussion

There are numerous records of *Choanotaenia crassiscolex* in the literature and some comprehensive descriptions have been produced (for example Lewis, 1964 and Vaucher, 1971). A detailed study of the anatomy of this cestode was therefore unnecessary. A comparison of measurements made in the current investigation and those recorded by Vaucher (Table 3.2) show that the species found may be positively identified as *C.crassiscolex*.

[1] includes length of rostellar sac

Due the low prevalence of *Choanotaenia hepatica* the existing descriptions are based on a small amount of material, in fact the original description was based on only a few immature specimens. This may help to explain the variability between the descriptions (Table 3.4). The present description confirms that the number of rostellar hooks is 46 as recorded by Baer (1932) and not 48 as suggested by Zarnowski (1955). The arrangement of the hooks was found to be in two irregular rows as stated by Vaucher (1971).

Apart from the number of rostellar hooks the current description agrees strongly with that given by Zarnowski (1955).

The only previous record of this species in Britain is by Lewis (1987).

Family Hymenolepididae Fuhrmann, 1907.

Eight species belonging to this family were found in the present study. The following six species have scolices armed with rostellar hooks: *Hymenolepis furcata, H.jacutensis, H.prolifer, H.schaldybini, H.scutigera* and *H.singularis*; the remaining two species, *H.diaphana* and *H.infirma* had unarmed scolices.

Hymenolepids with Armed Scolices

H.furcata is found in the intestine of both *Sorex araneus* and *S.minutus*. Mature specimens are large compared with other cestodes found in shrews. The rostellar hooks are similar in size and shape to those found by previous authors. The number of hooks is variable (23-29); most other cestodes found in the present study had a fixed number. The testes, which stain well with paracarmine were arranged in a triangle, one of them being on the poral, and the other two on the aporal side of the proglottid. Morphometric data for this species are compared with measurements made by Vaucher (1971) (Table 3.5). Some dimensions (scolex length and width, rostellum width, sucker length and width, and egg length and width) were slightly larger in the present study, but differences are not taxonomically significant.

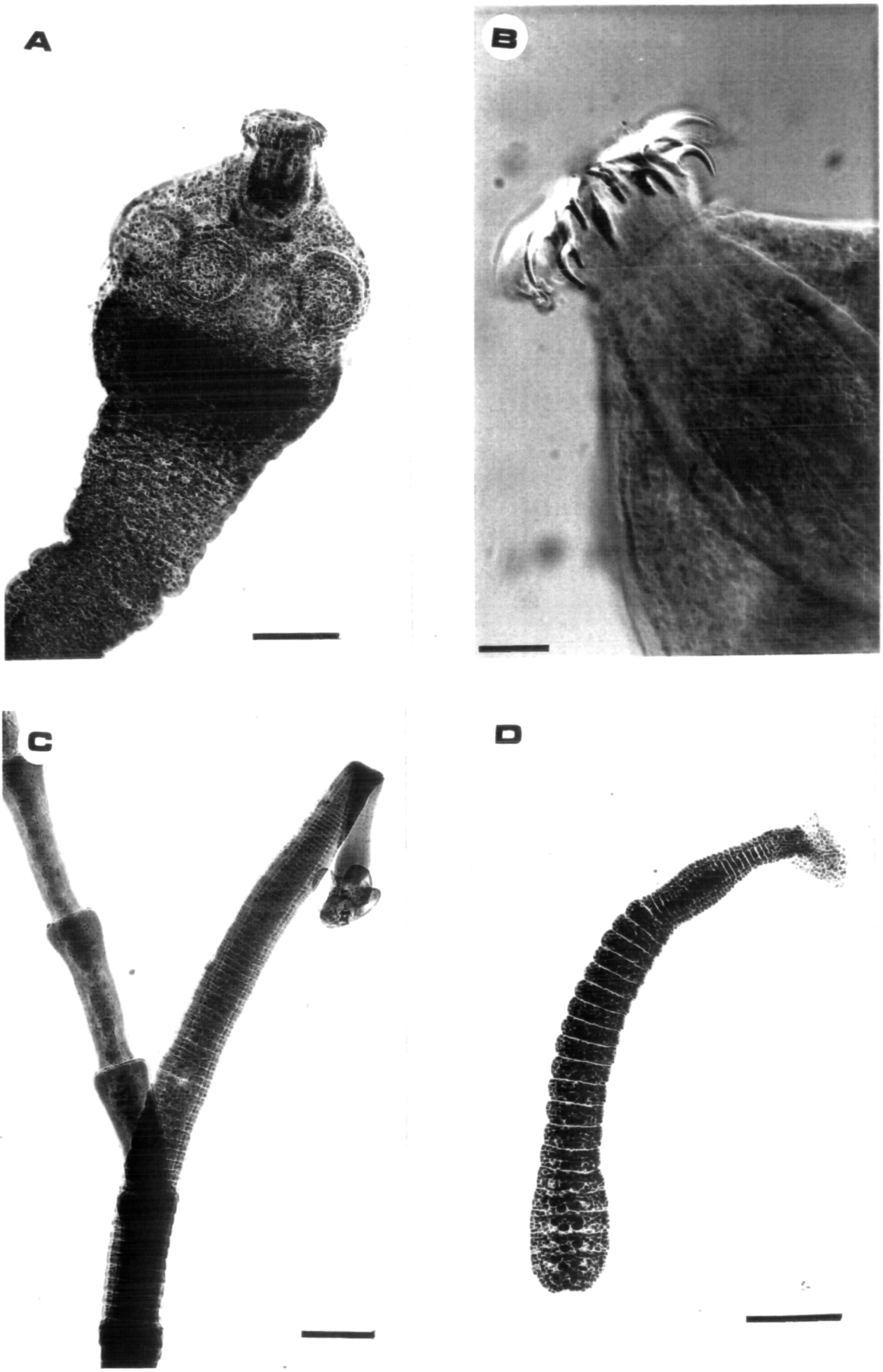
Hymenolepis jacutensis is found in the intestine of both species of shrews. The

rostellum is armed with about 20 hooks of varying size, arranged in a single irregular circle (Plate 3.1, Figure 3.1). A comparison with data obtained by Brendow (1969) and Vaucher (1971) (Table 3.6) shows that the specimens obtained in the present study were more similar to those of Brendow than the measurements made by Vaucher. This is especially evident in the scolex length and the dimensions of the rostellum. The rostellar sac has been measured for the first time in the present study.

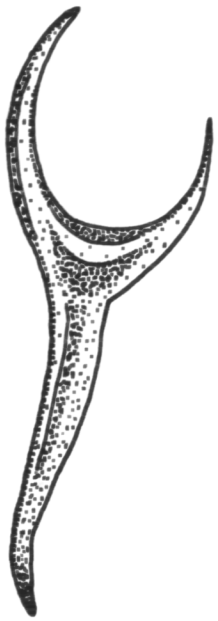
Only two specimens of the minute cestode *Hymenolepis prolifer* were examined due to a scarcity of material. The dimensions of the specimens found in the present study were similar to those of previous authors (Table 3.7) although there was considerable variation between measurements made by different authors. The characteristic feature of this species is the large number of tiny rostellar hooks present. The dimensions of the eggs of *H.prolifer* are recorded for the first time.

Hymenolepis schaldybini and *H.singularis* are morphologically similar in many respects. In both species the immature proglottids were short in comparison to their width, the three testes were arranged in a line and there were ten rostellar hooks. These were superficially similar in shape, but not in size. However, the hooks of *H.singularis* possess a flattened portion on the handle which is not present in those of *H.schaldybini*. Despite their morphological similarities, the two species are easily distinguishable on the basis of measurements of the scolex and its rostellum, suckers and hooks which are larger in *H.singularis* (Tables 3.8, 3.9; Vaucher, 1971).

Specimens of *Hymenolepis scutigera* are thin, string-like tapeworms with gravid proglottids which are about five times as long as they are wide (Plate 3.1). The rostellar hooks are very closely packed together when the rostellum is invaginated. When separated from the worm these hooks are seen to have a most characteristic shape, (Figure 3.1, Dujardin, 1845 and Vaucher, 1971). The three testes are arranged in a line. Morphometric data on *H.scutigera* are given in Table 3.10.

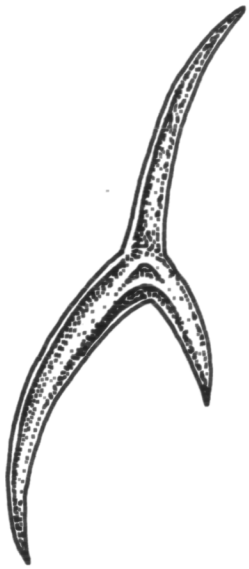

A
B
C
D

(A)



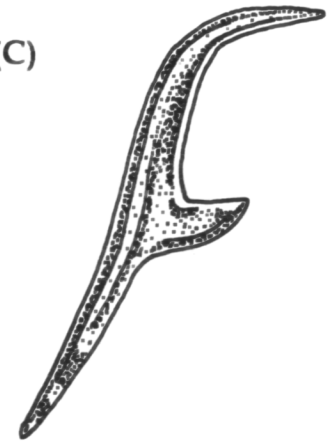
= 7μm

(B)



= 7μm

(C)



= 7μm

Table 3.5. Morphometric data on *Hymenolepis furcata* (present Study).

Characteristic	No. examined	Range	mean	S.D.	After Vaucher (1971)
Strobila length/mm	5	3.20-6.95	5.0	1.551	5.40
Max width/mm	3	0.950-1.228	1.122	0.146	1.300
Scolex width	5	317-457	380	57.7	174-279
Scolex length	4	238-349	328	61.1	192-320
Rostellum length	4	62-113	94	22.3	64-100
Rostellum width	4	100-133	111	15.3	58-73
Sucker length	15	80-127	102	14.0	59-111
Sucker width	15	74-123	97	15.1	48-88
No. of hooks	6	23-29	26	2.2	23-30
Hook length	11	23-27	24	1.0	23-27
Egg length	19	40-50	45	2.6	37-45
Egg width	19	29-39	33	2.9	27-34

(All measurements in μm unless otherwise stated.)

Table 3.6. Measurements of *Hymenolepis jacutensis* and a comparison with previous work.

Characteristic	Brendow (1969)	Vaucher (1971)	Present study
Length/cm	6.1	-	2.7
Max width/mm	-	0.900	1.452
Scolex length	324-540	180	311
Scolex width	312-516	c.240	227-289
Rostellum length	105-135	64	40-117
Rostellum width	63-84	41	62-97
Rostellar sac length	-	-	214-262
Rostellar sac width	-	-	107-125
Sucker length	240	107-118	131-191
Sucker width	150	66-84	80-104
No. of hooks	22	19-22	c.20
Hook length	29-33 & 48-53	29-50	25-46

(All measurements in μm unless otherwise stated.)

Table 3.7. Morphometric data on *Hymenolepis prolifer* and a comparison with previous work.

Characteristic	Zarnowski (1955)	Rybicka (1958)	Baer & Della Santa (1960)	Vaucher (1971)	Present study
Strobila length	350-600	670-900	270-548	400-1100	281-625
Strobila width	100-142	120-175	90-137	130-200	70-158
Scolex length	-	114-160	128-137	69-160	87-188
Scolex width	100-117	90-145	128-137	105-160	97-210
Rostellum length	40-46	40-43	27-34	19-37	27-120
Rostellum width	22-24	44-52	-	28-39	24-111
Sucker length	40-48	50-65	37-55	39-68	40-51
Sucker width	51-55	50-65	37-55	34-64	32-51
Egg length	-	-	-	-	43-45
Egg width	-	-	-	-	34-36
Oncosphere length	30-33	23-26	18-20	22-26	28
Oncosphere width	19-22	-	14-17	16-19	21
Hexacanth hook length	10-11	9	9-10	9-10	9-10

(All measurements in μm.)

Table 3.8. Morphometric data on *Hymenolepis schaldybini* (present study).

Characteristic	No. examined	Range	mean	S.D.
Strobila length/mm	4	7.200-12.600	9.344	2.301
Strobila width	6	168-448	335	103.5
Scolex length[1]	4	211-274	254	28.8
Scolex width	6	271-469	328	72.7
Rostellum length	3	57-141	91	44.3
Rostellum width	9	64-107	94	13.5
Sucker length	9	80-107	95	8.8
Sucker width	9	74-100	84	9.7
No. of hooks	-	10-10	10	-
Hook length	17	34-40	37	2.1
Egg length	4	77-97	83	10.5
Egg width	4	47-86	67	15.8
Emb. length	4	35-39	37	1.6
Emb. width	4	27-32	30	2.1
Oncosphere hook length	4	8-10	9.6	0.8

(All measurements in µm unless otherwise stated.)

[1] rostellum invaginated

Table 3.9. Morphometric data on *Hymenolepis singularis* (present study).

Characteristic	No. examined	Range	mean	S.D.
Strobila width	6	141-302	203	68.5
Scolex length	8	261-352	311	32.8
Scolex width	8	305-429	353	50.4
Rostellum length	4	70-89	77	8.2
Rostellum width	10	117-204	154	30.1
Sucker length	6	124-151	137	10.2
Sucker width	6	117-147	131	10.4
No. of hooks	-	10-10	10	-
Hook length	13	54-64	60	2.3
Egg length	5	87-97	92	4.0
Egg width	5	59-80	66	8.3
Emb. length	5	45-50	48	1.9
Emb. width	5	35-39	37	1.8

(All measurements in μm.)

Table 3.10. Morphometric data on *Hymenolepis scutigera* (present study).

Characteristic	No. examined	Range	mean	S.D.
Length/mm	1	-	20	-
Strobila width	1	-	94	-
Scolex length	9	151-227	196	30.0
Scolex width	9	150-215	188	23.6
Rostellum length	2	91-116	103	-
Rostellum width	2	47-57	52	-
Sucker length	4	67-100	86	11.8
Sucker width	4	53-57	55	1.6
No. of hooks	-	10-10	10	-
Hook length	7	32-40	34	2.7
Egg length	11	33-57	46	9.3
Egg width	11	27-52	39	9.5

(All measurements in μm unless otherwise stated.)

Discussion

In 1971 Claude Vaucher published the results of a comprehensive study of the anatomy of the cestodes of European Soricidae. All the species of cestodes recorded in the present investigation are described by Vaucher and, apart from the two species of *Choanotaenia*, belong to the genus *Hymenolepis*.

As Vaucher pointed out, *Hymenolepis* has been subdivided into a number of genera by previous authors. However, their classifications were usually based on a review of the literature rather than an examination of the actual species themselves. Thus in some cases taxonomy has been based on incorrect descriptions.

Since he was unable to find a satisfactory method of subdividing the genus, Vaucher left all the species in the original genus of *Hymenolepis* Weinland, 1858.

The system of classification used by Vaucher has been followed in the current study.

The specimens of *Hymenolepis furcata* recovered in the present study show a strong similarity to those found by Vaucher (1971) (Table 3.5) and there is no doubt as to their identity. Although this species was fairly common in both *Sorex araneus* and *S.minutus* in the present investigation, records of *H.furcata* in the British literature are surprisingly rare. This may perhaps be attributed to incorrect identifications. Baylis (1928) records *Hymenolepis uncinata* and *H.pistillum* in *S.araneus*, but does not give a description. These two species are considered by Vaucher (1971) to be specific to *Crocidura sp.* The shape of the rostellar hooks of these species is similar to that of *H.furcata*. However, the number of hooks of *H.uncinata* (16-19 compared to 23-29 in *H.furcata*) and the size of the hooks in *H.pistillum* (12-13μm compared to 23-27μm in *H.furcata*) clearly distinguish them.

James (1954) also (presumably incorrectly) recorded *H.uncinata* in a British specimen of *S.araneus*.

Sharpe (1964) recorded the following cestodes in *S.araneus*, none of which he described: *Choanotaenia crassiscolex, Hymenolepis scalaris, H.scutigera, H.singularis* and *H.spinulosa*. His record of *H.singularis* may actually refer to *H.schaldybini* and *H.spinulosa* may actually have been *H.jacutensis* (see below). If this is the case then it may be inferred that Sharpe incorrectly identified *H.furcata* as *H.scalaris* since the prevalence data in Chapter 6 suggest that he would have been very likely to have encountered *H.furcata*.

There are only four previous records of *Hymenolepis jacutensis* in the literature. Vaucher (1971) synonymised the species *Skrjabinocanthus jacutensis* (Spassky & Morosov, 1959) and *Pseudoparadilepis ankeli* (Brendow, 1969) considering that the differences between them were due to geographical variation. Brendow (1969) placed the parasite in the family Dilepididae due to the presence of what he thought were two circles of hooks. However, Vaucher (1971) considered there to be only one irregular circle of hooks and therefore placed the species in the family Hymenolepidae due to the presence of three testes. Examination of the specimens

found in the present study showed Vaucher's observation to be correct.

The dimensions of the specimens found in the present study are closer to those of Brendow (1969) than to those of Vaucher (1971), but this is not thought to be significant since Vaucher compared specimens from Brendow's collection with his own specimens and considered them both to be the same species.

H.jacutensis has not previously been recorded in Britain. Cameron & Parnell (1933) found a cestode resembling *H.spinulosa* in *S.araneus*, but were unable to identify it due to its poor condition. Sharpe (1964) recorded the presence of *H.spinulosa* in *S.araneus*, but it is likely that he found *H.jacutensis* since this species has similar dimensions to *H.spinulosa* and also has an irregularly arranged crown of rostellar hooks. *H.jacutensis* could not have been confused with *H.spinulosa* in the present study since the hooks of this species are smaller and shaped differently to those found in the present investigation (Vaucher, 1971).

Hymenolepis prolifer was only recorded from one *S.araneus* (Chapter 6) and not recorded at all from *S.minutus*. Hence the material available for morphological studies was very limited and measurements were confined to two specimens one of which was damaged by flattening under a coverslip; this accounts for some of the measurements being larger than those recorded by previous authors (Table 3.7). The specimens found in the present study have similar dimensions to those found previously although Table 3.7 shows that there is a large amount of variability in the measurements made by different authors. Since *H.prolifer* is the only species of cestode from shrews known to have a rostellum armed with minute hooks the specimens found may be assigned to this species.

H.prolifer has not previously been recorded from Britain. Vaucher (1971) suggests that the record by Lewis (1968) of *Protogynella blarinae* Jones 1943 was in fact *H.prolifer*. However, since *H.infirma* was much more common than *H.prolifer* in the present study (Chapter 6), it is likely that *H.infirma* was the species found by Lewis. *H.prolifer* may be readily distinguished from *P.blarinae* by the presence of hooks on the rostellum of the former; *H.infirma* is readily distinguished from *P.blarinae* by the greater number of proglottids in the latter.

Hymenolepis schaldybini was first described comparatively recently by Spassky (1947) who placed it in a new genus, *Neoskrjabinolepis* because of the unusual development of the gravid proglottids. He found that a group of proglottids would become detached from the rest of the strobila and the uteri of these proglottids would then unite to form a single capsule.

The specimens found in the present study have been identified as *H.schaldybini* by comparing them with the original description by Spassky (1947) and the redescription by Vaucher (1971). *H.schaldybini* was very prevalent in both *S.araneus* and *S.minutus* (Chapter 6) so it is very surprising that there are so few records of this species in the literature. The reason for this is likely to be incorrect identification. Despite the fact that there is very little similarity between them, *H.schaldybini* has certainly previously been confused with *H.scalaris*. The drawing of the rostellar hook of '*H.scalaris*' by Vaucher & Hunkeler (1967) is clearly that of *H.schaldybini* so it is conceivable that records of *H.scalaris* by Baylis (1928), Stammer (1955), Lewis (1964, 1968) were the result of incorrect identification of *H.schaldybini*.

H.schaldybini is very similar to *H.singularis*, the main differences being in the dimensions of the scolex. A simple method of separating the two species is by the size of the rostellar hooks: 34-40μm in *H.schaldybini* and 54-64μm in *H.singularis*. In his original description of *H.singularis*, Cholodkowsky (1912) correctly determined the rostellar hooks to be 55μm in length. However, Baylis (1934) assumed that this figure was due to a typographical error and that the correct figure was in fact 35μm, since his own specimens had hooks of between 34 and 40μm in length. Pojmanska (1957) records *Neoskrjabinolepis singularis* with rostellar hooks 37-42μm in length. The other morphological measurements which she made suggest that her specimens were in fact *H.schaldybini* rather than *H.singularis*. Since *H.schaldybini* is more prevalent than *H.singularis* (Chapter 6), the above authors and others are more likely to have encountered it. However, due to the comparatively recent description of *H.schaldybini*, 35 years after that of *H.singularis*, published in Russian and therefore not easily accessible, the parasites were identified as *H.singularis*.

The present author is the first to identify correctly *H.schaldybini* in British

shrews.

Hymenolepis singularis has been recorded many times in the literature, but as discussed above the identification in several of these cases is in doubt.

Zarnowski (1955) considered *H.singularis* and *H.schaldybini* to be the same species in the which the dimensions of the scolex and hooks were highly variable. However, this is unlikely because if this were the case then either there would be two distinct subspecies separated from each other by geographical distribution or else there would be a continuous variation from small hooks and scolices to large ones. *H.schaldybini* and *H.singularis* are clearly two separate species, *H.schaldybini* having hooks of 34-40μm while those of *H.singularis* range from 54 to 64μm, since they were found in the same host individual on several occasions (personal observation). In addition to the large difference in hook size the hooks also differ in shape.

The specimens of *Hymenolepis scutigera* recovered in the present study could be readily identified with those described by Vaucher (1971) as the shape and size of the hooks and the very elongated proglottids are characteristic of this species.

The dimensions of the eggs in the present study were found to be smaller than those described by Vaucher (1971).

H.scutigera was relatively common in the present study and this is borne out by the number of records of this parasite in the literature.

Hymenolepids with Unarmed Scolices

Hymenolepis diaphana is very distinctive in that the strobila is divided into three or four sections; the proglottids within each section are at the same stage of development (Plate 3.1). In the other species of cestodes found, the state of maturity of the proglottids increases gradually along the length of the strobila. There are three testes per proglottid (diagnostic of the Hymenolepididae), two poral and one aporal, but since the two poral testes are arranged one above the other, each proglottid appears to contain only two testes unless examined carefully. The scolex possesses a rudimentary rostellum which is not armed with hooks. Morphometric data on *H.diaphana* are shown in Table 3.11. The present specimens could be identified as *H.diaphana* rather than the morphologically similar *H.tripartita* by the dimensions of the scolex and number of proglottids in each section (Table 3.12).

Table 3.11. Morphometric data on *Hymenolepis diaphana* (present study).

Characteristic	No. examined	Range	mean	S.D.
Strobila length/mm	5	1.140-2.470	1.738	0.506
Max width	9	132-379	242	88.0
Scolex length	7	121-243	168	38.7
Scolex width	7	114-298	191	61.9
Sucker length	6	94-116	105	7.7
Sucker width	4	50-75	62	10.6
Cirrus length	2	50-64	57	-
No. of proglottids per section	9	5-29	19	7.4

(All measurements in μm unless otherwise stated.)

Table 3.12. Comparison of the morphology of the present specimens of *Hymenolepis diaphana* with that of *H.tripartita* and *H.diaphana* after Vaucher (1971).

Characteristic	*H.tripartita* (Vaucher, 1971)	*H.diaphana* (Vaucher, 1971)	*H.diaphana* (Present study)
Scolex width	160-260	114-197	114-298
Sucker length	84-178	64-164	94-116
No. of proglottids per section	8-12	15-40	5-29

Hymenolepis infirma is a very small cestode similar in size to *H.prolifer*, but easily distinguishable by the lack of a rostellum and the smaller number of proglottids (9-13 compared to at least 20). Morphometric data on this tapeworm are shown in Table 3.13. The length of the hooks of the oncosphere (13-16μm) is recorded for the first time. The eggs and embryophore are larger than previously recorded (Table 3.14) although the embryophore was similar in size to that recorded by Zarnowski (1955) and Mas Coma & Gallego (1975).

Table 3.13. Morphometric data on *H.infirma* (present study).

Characteristic	No. examined	Range	mean	S.D.
Strobila length	7	343-697	448	115.8
Strobila width	3	65-82	75	8.7
Scolex length	4	81-146	106	30.2
Scolex width	4	79-208	125	57.2
Sucker length	4	50-70	62	8.5
Sucker width	4	27-42	37	6.8
No. of proglottids	5	9-13	11	1.6
Egg length	3	69-129	90	33.8
Egg width	3	46-92	62	25.7
Embryophore length	6	28-31	30	0.9
Embryophore width	6	20-27	22	2.4
Oncosphere hook length	4	13-16	15	1.7

(All measurements in μm.)

Table 3.14. Morphometric data on *Hymenolepis infirma* compared with previous studies.

Characteristic	Zarnowski (1955)	Vaucher (1971)	Mas Coma & Gallego (1975)	Present study
Strobila length	308-605	?-1300	349-502	343-697
Strobila width	93-175	?-140	83-104	65-82
Scolex length	-	73-96	65-94	81-145
Scolex width	105-162	96-114	92-115	79-208
Sucker length	75-107	45-54	50-72	50-70
Sucker width	50-78	34-45	36-50	27-42
No. of proglottids	7-15	-	8-13	9-13
Egg length	49-55	48-64	49-58	69-129
Egg width	28-36	30-39	29-36	46-92
Embryophore length	32-34	-	31-39	28-31
Embryophore width	17-20	-	16-22	20-27

(All measurements in μm.)

Discussion

A distinctive feature of *Hymenolepis diaphana* is the division of the strobila into groups of proglottids, all proglottids in a group having the same level of maturity. This characteristic is also shared by *H.tripartita*. Vaucher (1971) states that *H.diaphana* can be distinguished from *H.tripartita* by the smaller diameter of the scolex, shorter suckers and larger number of proglottids per section of strobila. The specimens found in the present study could not be clearly identified as *H.diaphana* on the basis of measurements of the scolex, but Vaucher (1971) states that these measurements vary considerably depending on the state of contractions of the worms. The number of proglottids per section of the strobila corresponded to the number found in *H.diaphana* rather than in *H.tripartita* (Table 3.12). Vaucher

(1971) also stated that the two species differed in the development of the ovary and uterus and that the gravid proglottids of *H.tripartita* are separated from the rest of the strobila by some very elongated sterile segments with no trace of organs. The parasites found in the present study therefore clearly belong to *H.diaphana* rather than *H.tripartita*.

The only previous British record of *H.diaphana* in the literature is by Baylis (1928). Although Baylis gives no description of the parasite, it is likely that he did indeed find *H.diaphana* because it was found to be fairly prevalent in the present investigation and is distinctive enough not to be confused with any other species except for *H.tripartita*.

Although the parasite has been recorded in *S.minutus* elsewhere, this is the first record of *H.diaphana* occurring in this species in Britain.

Hymenolepis infirma was readily identified by its small size and the absence of a rostellum. The measurements of morphological features which were made are in agreement with those of previous authors (Table 3.14). The only discrepancy is the difference in egg size, the eggs of the present specimens being larger than those previously recorded. However, the differences only applied to the size of the outer egg capsule; the size of the embryophores was in agreement with measurements made by previous authors.

The number of previous records of *H.infirma* is very limited, this may be due to its small size making it difficult to detect, and its low prevalence. *H.infirma* has not previously been recorded in Britain, but Lewis (1964, 1968) recorded *Protogynella blarinae* Jones 1943 in *S.araneus* and it seems likely that he actually found *H.infirma* since the two species are superficially similar.

The measurements of cestode eggs recorded in the present study were often appreciably different from those recorded by Vaucher (1971). This may be attributed to the different methods of preparation of the material which was examined. In the present study measurements were usually made on fresh material, while Vaucher fixed his material in 10% formalin. In general the egg measurements made in the present study are larger than those made by Vaucher

and this is to be expected with fresh material (Vaucher 1971).

As described above there have been a series of incorrect identifications of cestodes found in *S.araneus* and *S.minutus* in previous studies. A large proportion of these errors can be attributed to incorrect identification of the host species by Dujardin (1845). Dujardin accurately described a number of new species of cestodes from two species of shrews which he identified as *'Sorex araneus'* and *'Sorex tetragonurus'*. Vaucher (1971) considers that Dujardin mistook *Crocidura russula* Hermann for *S.araneus* since the two species were considered synonymous by some mammalogists at the time. This hypothesis is supported by the fact that Dujardin's descriptions of *'Taenia pistillum* n.sp.', *'Taenia scalaris* n.sp.' and *'Taenia tiara* n.sp.' agree with those of Vaucher (1971) for *Hymenolepis pistillum* (Duj. 1845), *H.scalaris* (Duj. 1845) and *H.tiara* (Duj. 1845). Vaucher (1971) stated that these species were only found in shrews of the genus *Crocidura.*

Sorex tetragonurus was used as a synonym for *S.araneus* (Corbet & Harris, 1982). Evidence that Dujardin (1845) indeed used this name for *S.araneus* was obtained by comparing the description of *'Taenia scutigera* n.sp.' with that of *Hymenolepis scutigera* in the present study. Thus *'Taenia scutigera'* described by Dujardin (1845) from *'Sorex tetragonurus'* is the same species of helminth as *Hymenolepis scutigera* described from *S.araneus* in the present study.

An up-to-date list of the species of cestodes recorded in *Sorex araneus* and *S.minutus* in Europe has been compiled and is given below. All known synonyms of species found in the present study are also listed.

A key to the identification of the cestodes recovered from *S.araneus* and *S.minutus* in the present study is also given below.

List of Cestodes (with synonyms) Found in European *S.araneus* and *S.minutus* with a List of Records of Occurrence of Those Species Found in The Present study.

Family Dilepididae Fuhrmann 1907

Choanotaenia crassiscolex (Linstow 1890) *

Records in the literature:
Soltys 1952; Soltys 1954; James 1954; Stammer 1955; Zarnowski 1955; Pojmanska 1957; Kisielewska 1958a; Rawson & Rigby 1960; Kisielewska 1961; Kisielewska 1963; Kisielewska & Prokopic 1963; Lewis 1964; Rybicka 1964; Sharpe 1964; Vaucher & Hunkeler 1967; Wahl 1967; Lewis 1968; Vaucher 1971; Jourdane 1971; Canning et al. 1973; Mas Coma & Gallego 1975; Grainger & Fairley 1978; Murai & Meszaros 1984; Lewis 1987; Haukisalmi 1989.

Synonyms and records:
Amoebotaenia subterranea Cholodkowsky 1906.
Choanotaenia soricina (Cholod. 1906) Baylis 1934.
Molluscotaenia crassiscolex (Linstow 1890) Spassky & Andreiko 1970; Andreiko 1970.
Monopylidium scutigerum (Dujardin 1845) Baer 1928, Baer 1932.
Monopylidium soricinum (Cholod. 1906) Baer 1928.
Monopylidium subterranea (Cholod. 1906) Baer 1928.
Taenia crassiscolex Linstow 1890.

Choanotaenia hepatica (Baer 1932) Soltys 1952 *

Records:
Soltys 1952; Stammer 1955; Zarnowski 1955; Kisielewska & Prokopic 1963;

Vaucher & Hunkeler 1967; Vaucher 1971; Lewis 1987; Haukisalmi 1989.

Synonyms and records:
Monopylidium hepaticum Baer 1932.
Prochoanotania hepatica (Baer 1932) Spassky 1965; Andreiko 1973.

Dilepis undula (Schrank 1788) larva.

Family Hymenolepididae Fuhrmann 1907

Hymenolepis diaphana Cholodkowsky 1906 *

Records:
Baylis 1928; Vaucher & Hunkeler 1967; Vaucher 1971; Jourdane 1971; Mas Coma & Gallego 1975.

Synonyms and records:
Dicranotaenia diaphana (Cholod. 1906) Skrjabin & Mathevessjan 1948.
Ditestolepis diaphana (Cholod. 1906) Soltys 1952; Soltys 1954; Stammer 1955; Rybicka 1964: Andreiko 1973.
Neoskrjabinolepis diaphana (Cholod. 1906) Kobulej 1953.
Soricinia diaphana (Cholod. 1906) Zarnowski 1955; Prokopic 1959?; Kisielewska 1960b; Kisielewska 1961; Kisielewska 1963b; Kisielewska & Prokopic 1963.
Spasskylepis ovaluteri Schaldybin 1964.

Hymenolepis furcata (Stieda 1862) Meggitt 1924. *

Records:
Baer 1925; Meggitt 1927; Johri 1934; Stammer 1955; Kisielewska 1959;

Kisielewska 1961; Sosnina 1961; Rybicka 1964; Vaucher & Hunkeler 1967; Vaucher 1971; Jourdane 1971; Andreiko 1973; Mas Coma & Gallego 1975; Murai & Meszaros 1984; Haukisalmi 1989.

Synonyms and records:
Hymenolepis pistillum (Duj. 1845) sensu Baylis 1928?; Wahl 1967?
Hymenolepis scalaris (Duj. 1845) sensu Sharpe 1964?
Hymenolepis uncinata (Stieda 1862) Sensu Baylis 1928?; Baer 1932; James 1954?; Andreiko 1973?
Lepidotras furcata (Stieda 1862) Cohn 1869.
Staphylocystis furcata (Stieda 1862) Zarnowski 1955; Pojmanska 1957; Prokopic 1959; Kisielewska 1963b; Kisielewska & Prokopic 1963; Lewis 1987; Rysavy 1989.
Taenia furcata Stieda 1862.
Weinlandia furcata Mayow 1925.

Hymenolepis globosoides (Soltys 1954)

Synonym:
Dicranotaenia globosoides Soltys 1954.

Hymenolepis infirma (Zarnowski 1955) Vaucher & Hunkeler 1967. *

Records:
Vaucher 1971; Mas Coma & Gallego 1975; Murai & Meszaros 1984; Haukisalmi 1989.

Synonyms and records:
Ditestolepis secunda Schaldybin 1964.
Insectivorolepis infirma Zarnowski 1955; Andreiko 1973.

Protogynella blarinae Jones 1943 sensu Lewis 1964, 1968?

Hymenolepis jacutensis (Spassky & Morosov 1959) Vaucher 1971. *

Records:
Jourdane 1971; Vaucher 1971.

Synonyms and records:
Hymenolepis spinulosa sensu Sharpe 1964?
Pseudoparadilepis ankeli Brendow 1969.
Skrjabinocanthus jacutensis Spassky & Morosov 1959.

Hymenolepis prolifer (Villot 1880) Vaucher & Hunkeler 1967. *

Records:
Vaucher 1971; Jourdane 1971; Haukisalmi 1989.

Synonyms and records:
Hymenolepis curiosa Stammer 1955.
Hymenolepis prolifera (Villot 1880) Stammer 1955.
Pseudodiorchis kampinosi Rybicka 1958, 1968.
Pseudodiorchis multispinosa Zarnowski 1955.
Pseudodiorchis prolifer Kisielewska 1961; Kisielewska 1963b.
Urocystis prolifer (Villot 1880) Andreiko 1973

Hymenolepis schaldybini (Spassky 1947) Vaucher 1971. *

Records:
Jourdane 1971; Vaucher 1971; Murai & Meszaros 1984; Haukisalmi 1989.

Synonyms and records:

Hymenolepis scalaris (Dujardin 1845) Baer 1932; Stammer 1955?; Lewis 1964?; Vaucher & Hunkeler 1967; Wahl 1967; Lewis 1968?.

Hymenolepis scutigera (Dujardin 1845) sensu Dollfus 1961.

Hymenolepis singularis (Cholodkowsky 1912) sensu Baylis 1928, 1934; Kobulej 1953; Zarnowski 1955; Prokopic 1956; Pojmanska 1957; Rybicka 1959, 1964; Kisielewska 1961; Sharpe 1964?

Neoskrjabinolepis schaldybini Spassky 1947.

Neoskrjabinolepis schaldybini Spassky 1947 sensu Schaldybin 1964; Andreiko 1973.

Neoskrjabinolepis singularis Pojmanska 1957.

Hymenolepis scutigera (Dujardin 1845) Baylis 1928. *

Records:

Baylis 1928; James 1954?; Stammer 1955; Zarnowski 1955; Pojmonska 1957; Prokopic 1959; Kisielewska 1961; Kisielewska 1963b; Sharpe 1964; Lewis 1964, 1968; Vaucher & Hunkeler 1967; Prokopic 1969; Vaucher 1971; Jourdane 1971; Smit 1974; Mas Coma & Gallego 1975; Grainger & Fairley 1978; Smit 1978; Murai & Meszaros 1984; Lewis 1987; Haukisalmi 1989.

Synonyms and records:

Hymenolepis toxometra Baer 1932.

Taenia scutigera Dujardin 1845.

Hymenolepis singularis Cholodkowsky 1912. *

Records:

Baer 1932?; Soltys 1952; Kobulej 1953; James 1954?; Soltys 1954; Stammer 1955; Prokopic 1957; Rybicka 1959; Prokopic 1959; Kisielewska 1961; Rybicka 1964;

Vaucher & Hunkeler 1967; Lewis 1968?; Vaucher 1971; Haukisalmi 1989.

Synonyms and records:
Dicranotaenia (Dicranotaenia) singularis (Cholod. 1912) Lopez-Neyra 1942.
Neoskrjabinolepis singularis (Cholod. 1912) Spassky 1947; Zarnowski 1955; Kisielewska 1963b; Kisielewska & Prokopic 1963; Andreiko 1973; Lewis 1987?

Hymenolepis spinulosa Cholodkowsky 1906.

Synonym:
Vigisolepis barboscolex Spassky 1949.

Hymenolepis stefanskii Zarnowski 1954.

Hymenolepis tripartita (Zarnowski 1955) Vaucher 1971.

Synonym:
Soricina tripartita Zarnowski 1955.

* = found in the present study.
? = the most likely identity of the helminth recorded by the author.

Key to the Cestode Parasites of *Sorex araneus* and *S.minutus* in Britain.

1.	Many testes per proglottid	*Choanotaenia*	2
	Three testes per proglottid	*Hymenolepis*	3
2.	Suckers oval, 46 rostellar hooks	*Choanotaenia hepatica*	
	Suckers elongated in vertical plane, 17-24 rostellar hooks	*Choanotaenia crassiscolex*	
3.	Rostellum vestigial or absent, if present not armed with rostellar hooks		4
	Rostellum armed with hooks		5
4.	Very small rostellum present, strobila divided into three or more sections in which all proglottids are at the same stage of development	*Hymenolepis diaphana*	
	No rostellum, strobila consists of 9-13 proglottids	*Hymenolepis infirma*	
5.	10 rostellar hooks present		6
	More than ten rostellar hooks present		8
6.	Gravid proglottids at least 5x as long as wide, arms of blade of rostellar hooks at right angles to guard	*Hymenolepis scutigera*	
	Gravid proglottids only about 2x as long as wide, arms of blade of rostellar hooks parallel with guard		7
7.	Rostellar hooks 34-40μm	*Hymenolepis schaldybini*	
	Rostellar hooks 54-64μm	*Hymenolepis singularis*	
8.	Strobila less than 2mm long, approximately 200 rostellar hooks	*Hymenolepis prolifer*	
	Strobila more than 2mm long, 20-30 rostellar hooks present		9
9.	Rostellar hooks all the same size and arranged in a regular row, suckers roughly oval in shape	*Hymenolepis furcata*	
	Rostellar hooks varying in size and arranged in an irregular row, suckers elongated	*Hymenolepis jacutensis*	

Digeneans

Family Brachylaemidae Joyeux & Foley 1930

Brachylaemus fulvus (Table 3.15, Plate 3.2) is found in the oesophagus and stomach of both *S.araneus* and *S.minutus*. The name "*fulvus*" refers to the yellowish coloration of the eggs. Other identificatory features include the position of the ovary, sandwiched between the testes in the posterior third of the body, and the prominent pharynx situated just beneath the oral sucker.

Family Dicrocoeliidae Odhner, 1910

Dicrocoelium soricis (Table 3.16, Plate 3.2) is found in the gall bladder and bile duct of both shrew species. *D.soricis* is elongate and about three times as long as *B.fulvus*, but only 1.4x as wide. The ventral sucker is large and the two testes are situated anterior to the ovary. The testes and ovary are located in the anterior half of the body.

Family Plagiorchidae Pratt, 1902

Opisthioglyphe sobelevi was found only in the sample of *S.araneus* obtained from Dungeness. This small pear-shaped digenean was recovered from the intestine. The body is covered in small spines, 8μm in length; the spines are more dense over the anterior two-thirds of the body. Morphometric data on this parasite compare very favourably with the description of *Opisthioglyphe (Neoglyphe) sobelevi* Schaldybin, 1953 (Table 3.17). A notable feature of this parasite when compared with the other species of the same genus is the very large cirrus (Plate 3.2); this large cirrus was also recorded by Schaldybin (1953). The pharynx and suckers of the present specimens are larger than recorded by the latter author. The dimensions of the testes are recorded for the first time in the present study.


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Table 3.15. Morphometric data on *Brachylaemus fulvus* (present study).

Characteristic	No. examined	Range	mean	S.D.
Worm length	4	2442-3762	3221	660.1
Width	4	469-858	690	162.1
Oral sucker length	7	220-297	266	28.3
Oral sucker width	7	220-284	254	23.2
Ventral sucker	7	245-315	295	26.9
Ventral sucker	7	237-315	290	27.1
Pharynx length	7	125-161	143	13.4
Pharynx width	7	158-211	180	17.3
Anterior testis length	4	188-285	232	43.2
Anterior testis width	4	174-265	222	40.5
Posterior testis length	4	174-275	232	46.9
Posterior testis width	4	168-273	222	48.4
Ovary length	4	168-231	190	29.3
Ovary width	4	141-183	163	21.1
Egg length	15	27-34	31	1.9
Egg width	15	15-17	16	0.8

(All measurements in μm.)

Table 3.16. Morphometric data on *Dicrocoelium soricis* (present study).

Characteristic	No. examined	Range	mean	S.D.
Worm length	2	9280-11524	10402	-
Width	2	924-1056	990	-
Oral sucker length	2	382-403	392	-
Oral sucker width	2	315-369	342	-
Ventral sucker length	2	552-581	567	-
Ventral sucker width	2	519-531	525	-
Anterior testis length	2	315-365	340	-
Anterior testis width	2	278-299	289	-
Posterior testis length	2	332-357	345	-
Posterior testis width	2	249-307	278	-
Ovary length	2	249-269	260	-
Ovary width	2	245-262	253	-
Egg length	15	35-40	38	1.8
Egg width	15	20-27	24	2.6

(All measurements in μm.)

Table 3.17. Morphometric data on *Opisthioglyphe sobolevi* and a comparison with *Opisthioglyphe (Neoglyphe) sobolevi* Schaldybin, 1953.

Characteristic	No. examined	Range	mean	S.D.	After Schaldybin (1953)
Worm length	6	589-917	721	97.3	573-690
Max width	6	307-452	370	56.0	340-408
Oral sucker length	4	60-79	73	9.1	57-69
Oral sucker width	4	79-96	89	7.6	57-69
Ventral sucker length	4	35-52	44	7.8	24-45
Ventral sucker width	4	35-54	44	8.0	24-45
Pharynx length	4	36-42	40	2.4	30
Pharynx width	4	32-42	38	4.5	30
Ant. testis length	3	67-104	89	19.7	-
Ant. testis width	3	285-307	298	11.4	-
Post. testis length	3	87-111	98	11.8	-
Post testis width	3	285-302	295	8.8	-
Ovary diameter	4	111-144	126	17.6	96-120
Cirrus pouch length	3	335-335	335	-	339
Egg length	13	30-38	33	2.4	33
Egg width	13	18-20	19	0.7	21

(All measurements in μm.)

Discussion

Brachylaemus fulvus is well described in the literature and has numerous synonyms as may be observed in the species lists below. Jourdane (1971) provided morphometric data from four authors. The dimensions of the present specimens compare favourably with these data although they are generally slightly larger. This can be attributed to the way the specimens were flattened in the present study.

There are comparatively few records of *Dicrocoelium soricis* in the literature and this can probably be attributed to the location of this helminth in the gall bladder which may have been overlooked as a possible site for parasitic infestation in many of the helminthological surveys undertaken on shrews. The taxonomy of *D.soricis* was reviewed by Lewis (1964) who also redescribed the species. The specimens described in the present study resembled those found by Lewis although several of the dimensions were larger than he recorded. Since only two specimens were used for morphometric analysis in the present study, the significance of these differences cannot be assessed.

Opisthioglyphe sobolevi

Kossack (1910) described *Neoglyphe locellus* from *Neomys fodiens*. The specimens described in the present study resemble those of Kossack, but differ in several dimensions especially in the sizes of the eggs and cirrus pouch.

In 1953 Schaldybin described *Opisthioglyphe (Neoglyphe) sobolevi* from *S.araneus* in the U.S.S.R., considering it to be a new species. The specimens found in the present study corresponded almost exactly with those of Schaldybin (Table 3.17). A similar digenean, *Opisthioglyphe soricis*, was recorded by Pojmonska (1956) from *S.araneus* in Poland; she later (Pojmanska, 1961) synonymised it with *O.sobolevi*. *O.sobolevi* was also recorded by Haukisalmi (1989) from *S.araneus* in Finland, but he did not give a description.

Genov (1978) revised the taxonomy of some trematodes from insectivores, synonymising *O.sobolevi* with *N.locellus*. He considered *O.sobolevi* to be a subspecies of *Neoglyphe locellus*, if this is the case then there should be a geographical separation between the two proposed subspecies. However, *O.sobolevi* has been recorded over a large geographical range (C.I.S.[1], Poland, Finland, Britain) sometimes in the same location as *Neoglyphe locellus* (Schaldybin, 1953). Thus it would appear that *O.sobolevi* and *N.locellus* are two separate species, *O.sobolevi* generally being found in *S.araneus* and *Neoglyphe locellus* in *N.fodiens*. It is not possible that *O.sobolevi* and *N.locellus* are two forms of the same species,

[1] Commonwealth of Independent States, formerly the U.S.S.R.

morphologically different in the two host species because Vaucher & Hunkeler (1967) recorded *O.sobolevi* in both *S.araneus* and *N.fodiens*, and Bock (1982) was able to experimentally infect both *S.araneus* and *Crocidura russula* with *O.locellus* (Bock uses the generic name *Opisthioglyphe* rather than *Neoglyphe*, but gives no reason for the change of name) and the morphology of the digeneans obtained from these two host species was that of *N.locellus* rather than *O.sobolevi*. It therefore appears that *O.sobolevi* is a distinct species from *N.locellus*, but the taxonomy of *Opisthioglyphe* and *Neoglyphe* obviously requires revision.

An up-to-date list of the species of digeneans recorded in *Sorex araneus* and *S.minutus* in Europe has been compiled and is given below. All known synonyms of species found in the present study are also listed.

Digeneans (with synonyms) found in European *S.araneus* and *S.minutus* with Records of Species Found in the Present Study

Family Brachylaemidae Joyeux & Foley 1930 syn. Harmostomidae Odhner 1912.

Brachylaemus fulvus (Dujardin 1843) Blanchard 1847. *

Records:
Dollfus 1935; Thomas 1953; Stammer 1955; Zarnowski 1960; Jourdane 1971, 1973; Andreiko 1973; Mas Coma & Gallego 1975; Meszaros et al. 1982; Haukisalmi 1989.

Synonyms and records:
Brachylaemus dujardini (Baer 1928) Baylis 1939; Thomas 1953.
Brachylaemus falva (Duj. 1843) Prokopic 1959
Brachylaemus oesophagei (Schaldybin 1953) Canning et al. 1973; Churchfield 1979.
Brachylaimus oesophagei Schaldybin 1953; Lewis 1964, 1968, 1969, 1987.
Brachylaima fulvum Dujardin 1843.
Brachylaime fulvum (Dujardin 1843) Pojmanska 1961.
Brachylaima fulvum (Duj. 1843) Kisielewska 1963b.
Distoma (Brachylaimus) migrans var. a Duj. 1845 sensu Baylis (1928).
Harmostomum (Harmostomum) dujardini Baer 1928.
Panopistus europaeus Soltys 1952, 1954.
Panopistus pricei Sinitsin 1931.

Cephalotrema minutum Baer 1943.

Pseudoleucochloridium soricis (Soltys 1952) Jourdane 1971.

Synonyms:

Leucochloridium skrjabini Schaldybin 1953.

Leucochloridium soricis Soltys 1952

Nephrotrema truncatum (Leuckart 1842) Jourdane 1971

Family Dicrocoeliidae Odhner 1910.

Corrigia soricis Jourdane, Theron & Gabrion 1980.

Dicrocoelium pellucidum Pojmanska 1956.

Dicrocoelium soricis (Diesing 1858) Dollfus, Callot & Desportes 1934. *

Records:

Joyeux & Baer 1936; Prokopic 1959; Lewis 1964, 1968; Grainger & Fairley 1978; Lewis 1987.

Synonyms and records:

Distoma soricis Stiles & Stanley 1932.

Distoma soricis araneus Stiles & Stanley 1932.

Distome (Dicrocoelium) de la Musaraigne musette Pontallié.

Distomum soricis Diesing 1858; Cobbold 1860; Stossich 1892; Braun 1893; Nicoll 1923.

Lyperosomum soricis Diesing 1858; Andreiko 1973; Chiriac & Popescu 1973.

Family Plagiorchidae Pratt 1902.

Opisthioglyphe exasperatum (Rudolphi 1819)

Synonyms:
Distoma exasperatum Rudolphi 1819.
Distoma rubens Dujardin 1845.
Distomum exasperatum (Rud. 1819) exempl. A & B Szidat 1928.
Echinostomum exasperatum (Rud. 1819) Nicoll 1923.
Echinostoma exasperatum (Rud. 1819) Nicoll 1931.
Opisthioglyphe (Rubenostrema) exasperatus (Rud. 1819) Prokopic 1959.
Plagiorchis exasperatus (Rud. 1819) Soltys 1952; Pojmanska 1952.
Plagiorchis microti Soltys 1949.

Opisthioglyphe opisthiovitellinus (Soltys 1954) Prokopic 1959.

Synonyms:
Distoma exasperatum exempl. C. Szidat 1928.
Plagiorchis opisthiovitellinus Soltys 1954.

Opisthioglyphe sobolevi Schaldybin 1953. *

Records:
Vaucher & Hunkeler 1967; Haukisalmi 1989.

Synonyms and Records:
Opisthioglyphe (Neoglyphe) sobolevi Schaldybin 1953.
Opisthioglyphe (Opisthioglyphe) sobolevi (Schaldybin 1953) Pojmanska 1961; Kisielewska 1963b.
Opisthioglyphe soricis Pojmanska 1956, 1957.

Family Nanophyetidae Dollfus 1939.

Skrjabinophyetus soricis Jourdane 1973.

* = found in the present study.

Nematodes

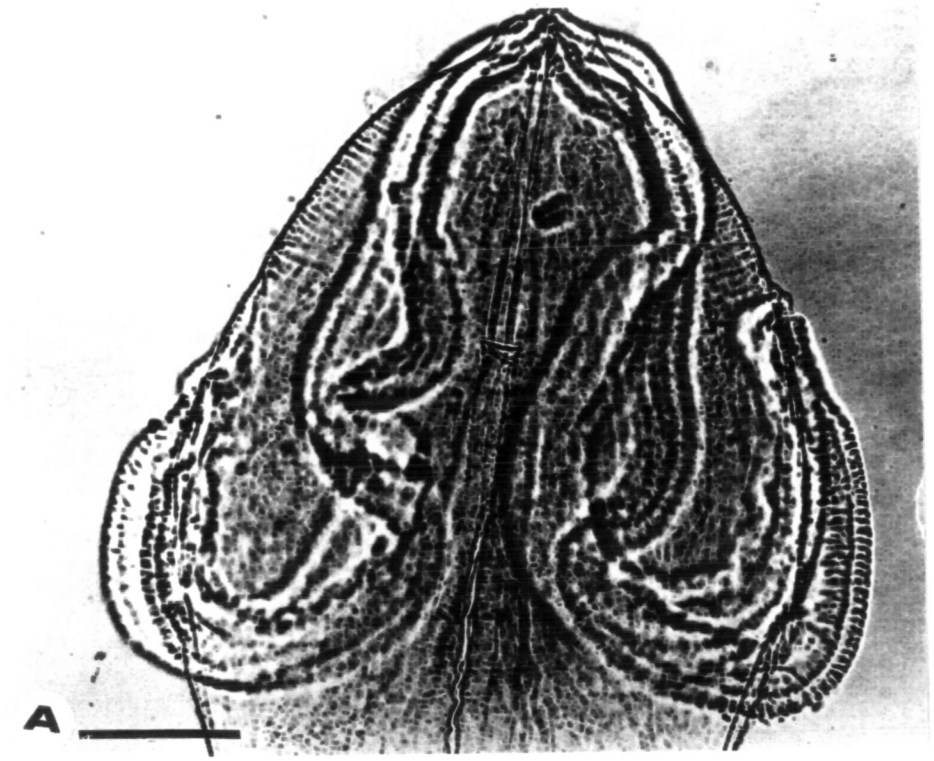
Family Acuariidae Seurat, 1913

Stammerinema soricis is found in the stomach of *S.araneus* and *S.minutus* with its head embedded in the stomach wall attached by its four thick cuticular ridges or 'cordons', diagnostic of the Acuariidae (Plate 3.3). The remainder of the body lies free in the lumen of the stomach. Another distinctive feature of *S.soricis* is the unequal size of the spicules (Plate 3.3). Morphometric data from the present study based on three males and one female is compared with measurements made by Tiner (1951) and Soltys (1952) (Tables 3.18, 3.19).

Table 3.18. Morphometric data on male *Stammerinema soricis* and a comparison with previous work.

Characteristic	Tiner (1951)	Soltys (1952)	Present study
Length	4930-7360	4500-6000	3565-7251
Oesophagus length	2400-3000	-	913-1671
Muscular oesophagus	300-390	-	291
Max width (body)	180-230	-	127-241
Head width	500	125	124-335
Short spicule length	140-160	160-170	135-149
Long spicule length	375-460	450-480	474-507

(All measurements in μm.)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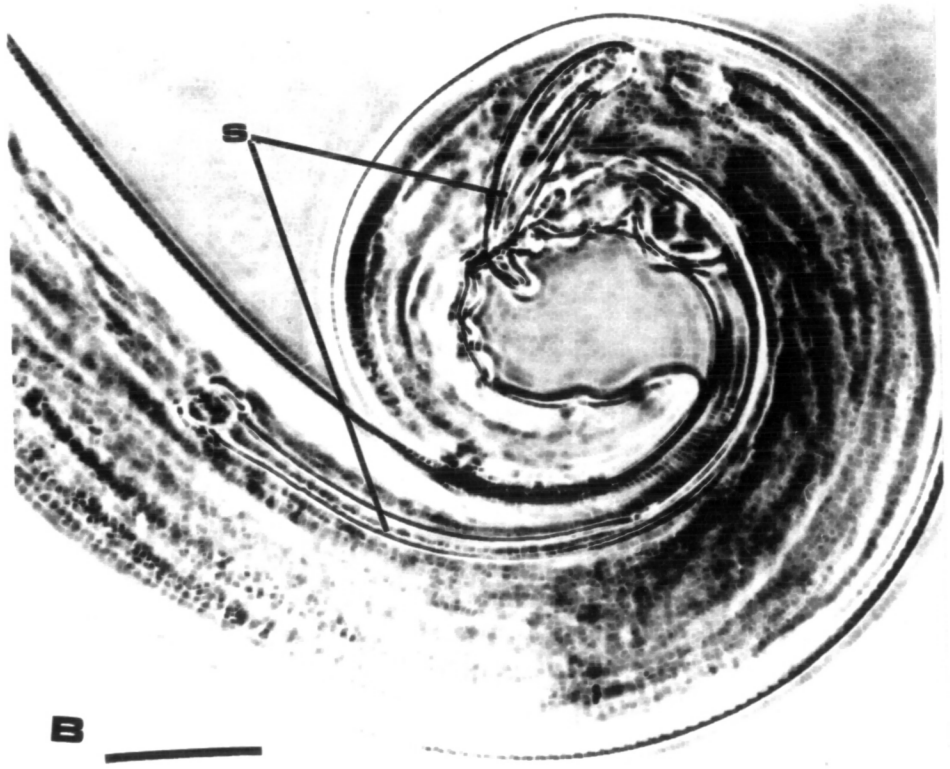
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Table 3.19. Data on female *Stammerinema soricis* and a comparison with previous work.

Characteristic	Tiner (1951)	Soltys (1952)	Present study
Length	7310-12700	1100-1400	6154
Oesophagus length	-	690	-
Musc. oesophagus	670	270	580
Max width (body)	300-400	-	188
Head width	1500-1600	490	193

(All measurements in μm.)

Discussion

Stammerinema soricis was first recorded by Tiner (1951) from *Sorex obscurus alascensis* under the synonym of *Dispharynx soricis*. Soltys (1952) recorded it in *S.araneus* in Poland as a new species which he named *Synhimatus rhopalocephalus*. The diagrams produced by the above authors are very similar and closely resemble the species found in the present study. Although there are some differences in the measurements of morphological features made by the two authors, these are probably not significant since Tables 3.18, 3.19 show that in some features the parasites found in the current investigation resemble those of Soltys, while in other features they are closer to those found by Tiner.

In his discussion of the taxonomy of the family Acuariidae, Osche (1955) synonymised *D.soricis* and *S.rhopalocephalus*, renaming the species *Stammerinema soricis*. The new genus *Stammerinema* was characterised by the swollen head region, weak development of cervical papillae, the presence of two rows of spines, and by having mammalian rather than avian hosts.

The first British record of *Stammerinema soricis* was by Churchfield (1979) who found the parasite in both *Sorex araneus* and *S.minutus*. British specimens obtained

by Dr J.W. Lewis from *S.araneus* at Rogate, Sussex and examined by the present author were also identified as *Stammerinema soricis*.

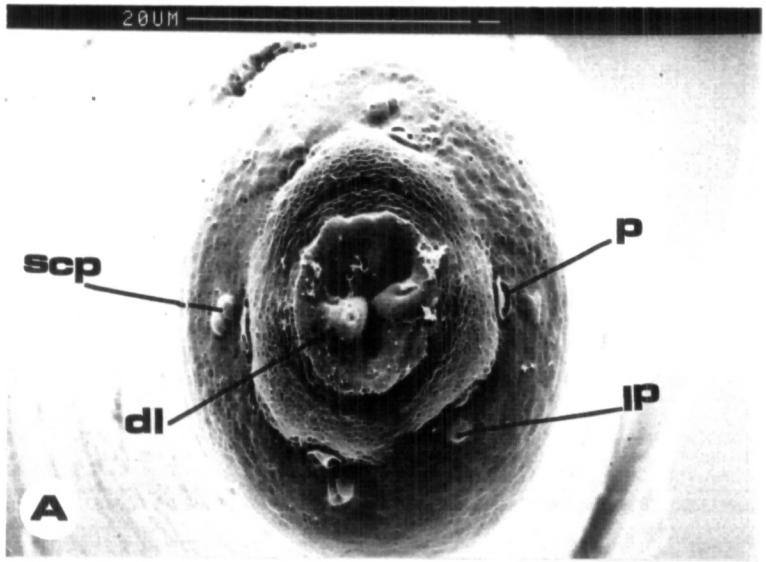
Family Angiostrongylidae

Stefanskostrongylus soricis was found in the lungs of *Sorex minutus* (in the bronchial tubes). Light microscopy identified this species with that described by Mas Coma (1977) (Tables 3.20, 3.21). The specimens obtained in the present study were slightly larger than those of Mas Coma, but were otherwise similar. The width at the anterior end and width at the vulva are recorded for the first time. Scanning electron microscopy (SEM) was used to obtain further information on the cephalic region and features of the cuticle. From an examination of fixed specimens it was not clear whether the excretory pore was anterior or posterior to the end of the oesophagus. In order to elucidate the relative positions of the oesophagus and excretory pore, five live specimens of *S.soricis* were each placed in a drop of saline on a microscope slide and covered with a coverslip. The specimens were observed immediately after putting them on the slides, and again 50 minutes later. At the start of the experiment the end of the oesophagus was anterior to the excretory pore in four of the specimens, but after 50 minutes the oesophagus had lengthened considerably, now extending beyond the excretory pore in all five specimens; this was presumably due to the pressure applied to the coverslip. It therefore appeared that the usual position of the excretory pore was just posterior to the end of the oesophagus, but that this depended on the state of contraction of the oesophagus.

When fresh specimens of infected shrews were examined the lungs were found to contain a large number of *S.soricis* larvae which had already hatched.

SEM Studies on *Stefanskostrongylus soricis*

SEM en face views of the cephalic region of two male specimens of *S.soricis* shows that there is a hexaradial symmetry of the papillae. There are four pairs of submedian cephalic papillae and two single lateral papillae. Associated with each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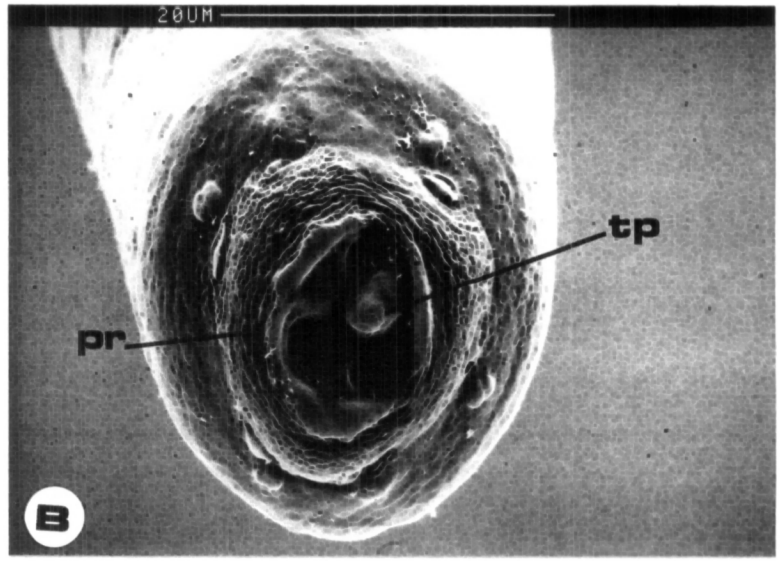
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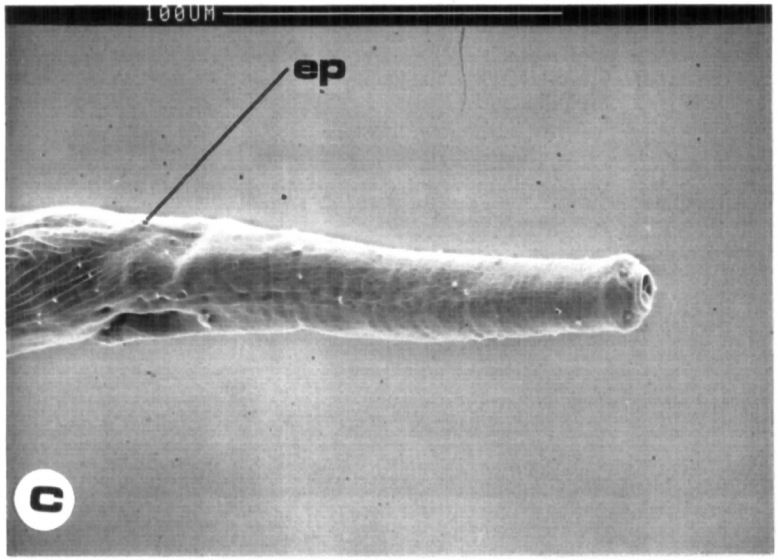
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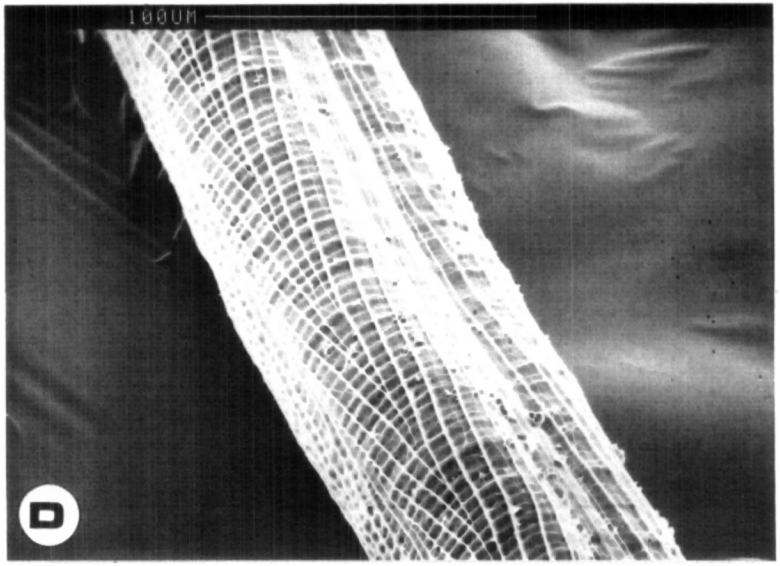
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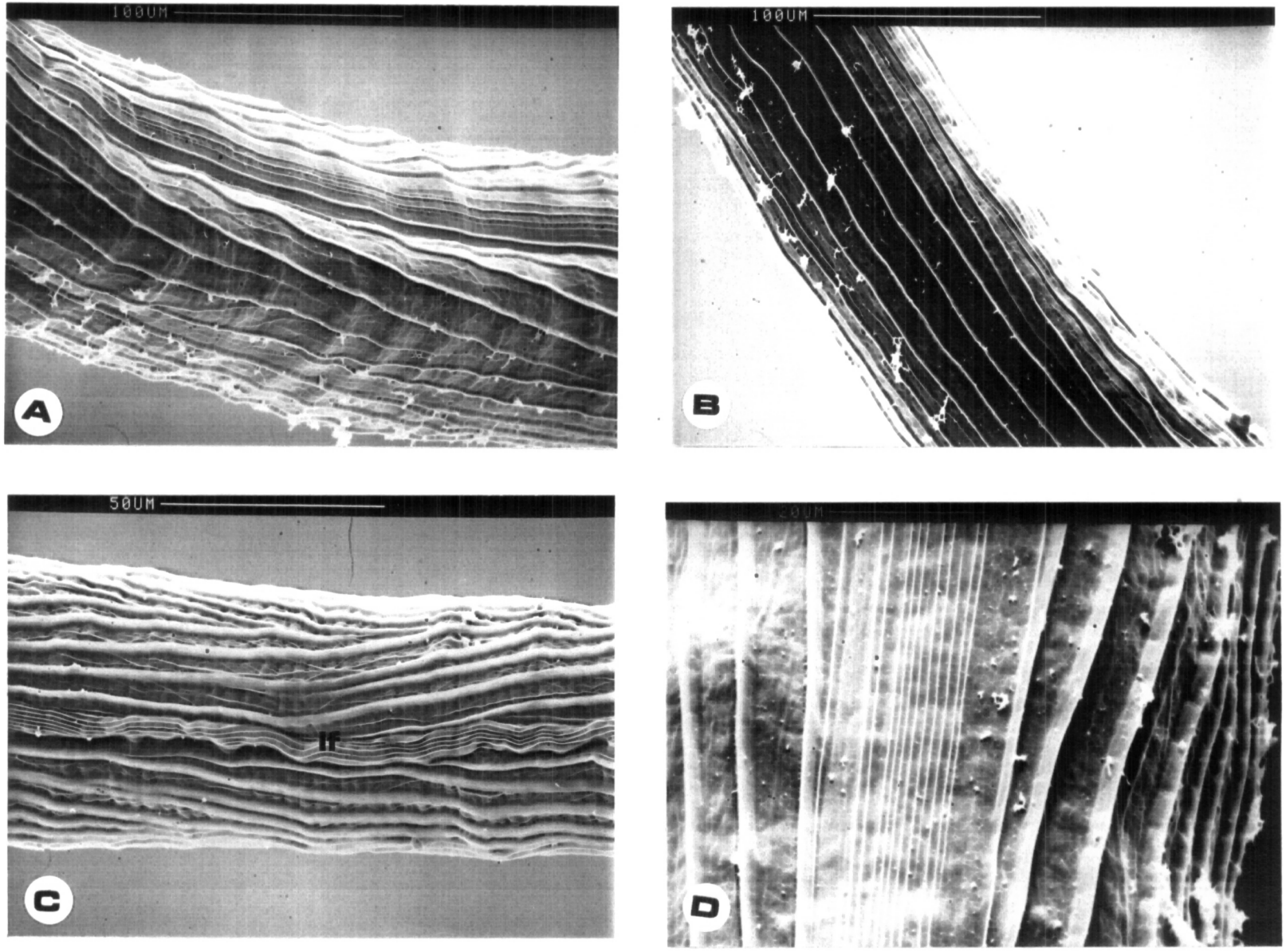
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papilla or pair of papillae there is a pair of slit-like pores. The mouth possesses three lips - a dorsal lip with a tooth-like projection and two sub-ventral lips with no such projection. The oral opening is surrounded by a raised cuticular area, the peribuccal ring (Plate 3.4).

SEM studies allowed a map of the cuticular surface to be built up. The extreme anterior end had no cuticular striations. The V-shaped transverse striations characteristic of *S.soricis* began just posterior to the excretory pore (Plate 3.4). Over the posterior quarter of the length of the worm the striations become longitudinal ones. The transition area and longitudinal striations are shown in Plate 3.5. The longitudinal striations finish about 200μm from the posterior end of the worm.

There are two lateral fields extending almost the whole length of the worm and an examination of several specimens showed that the lateral fields were made up of nine continuous parallel longitudinal striations (Plate 3.5).

Table 3.20. Morphometric data on male *Stefanskostrongylus soricis* compared with data obtained by Mas Coma, 1977.

Characteristic	No. examined	Range	Mean	S.D.	After Mas Coma (1977)
Worm length	10	3900-6300	5020	681	2593-4954
Oesophagus length	9	208-266	245	19.9	198-241
Excretory pore → apex	9	178-296	258	34.7	180-231
Maximum width	10	104-161	133	21.7	76-108
Width at ant. end	8	32-39	36	2.3	-
Length of bursa	10	85-111	102	7.3	86-94
Width of bursa	10	74-117	97	12.3	62-73
Spicule length	9	265-302	292	11.6	241-284
Gubernaculum length	10	42-52	47	3.4	40-47

(All measurements in μm.)

Table 3.21. Morphometric data on female *Stefanskostrongylus soricis* compared with data obtained by Mas Coma, 1977.

Characteristic	No. examined	Range	Mean	S.D.	After Mas Coma (1977)
Worm length	10	6400-10400	8060	1238	5301-8936
Oesophagus length	7	280-349	324	24.5	270-289
Excr. pore → apex	8	275-361	324	30.4	252-284
Maximum width	10	154-228	187	25.8	115-162
Width at ant. end	8	37-49	42	4.3	-
Width at vulva	2	117-131	124	-	-
Length of tail	10	60-79	68	6.0	65-79
Egg length	26	54-74	62	4.7	50-61
Egg width	26	32-49	38	4.0	29-36
Distance from vulva to posterior end	10	318-498	384	52.8	304-380

(All measurements in μm.)

Discussion

Stefanskostrongylus soricis was discovered in Poland by Soltys (1954) in the lungs of *S.minutus*, he placed the species in the genus *Angiostrongylus*. The description produced by Soltys was very brief although sufficient to equate his species with that found in the present study. Prokopic (1959) found *Stefanskostrongylus soricis* in the lungs of *S.minutus* in Czechoslovakia. He provided no description, but his drawings confirm his identification of the parasite.

In 1970 Drozdz revised the systematics of the genus *Angiostrongylus*, dividing it into three genera, one of which was *Stefanskostrongylus*. His basis for forming this new genus (using characteristics of the copulatory bursa) remains valid in the light of current knowledge. However, since his list of the generic characteristics for *Stefanskostrongylus* was based on incomplete descriptions (at

least in the case of *S.soricis*) this list requires updating. In addition to the shortness of the dorsal ray of the bursa which Drozdz describes as 'très longue', *S.soricis* also possesses a gubernaculum (absence of a gubernaculum was said by Drozdz to be a feature of *Stefanskostrongylus*, although not of essential taxonomic value). The presence of the gubernaculum was first recorded by Mas Coma (1977). He also found that in his specimens the vulva was further from the posterior end and the eggs were larger than stated in the description by Soltys (1952). These observations were confirmed in the present investigation.

There is strong agreement between the current study and the work of Mas Coma (Tables 3.20, 3.21).

The present investigation has added to the knowledge of *S.soricis* by providing information on the ultrastructure of the parasite (especially the cuticle) and on the development of the larvae. Observations on living specimens of *S.soricis* have shown that the usual position of the excretory pore is just posterior to the end of the oesophagus. Since the length of the oesophagus has been observed to change depending on the state of contraction of its musculature it is suggested that the distance from the excretory pore to the apex of the worm is a more reliable taxonomic feature than the length of the oesophagus.

S.soricis has not previously been recorded in Britain.

Family Ascarididae Baird, 1853

Larvae of *Porrocaecum* (L4) were found coiled up and encysted in the body cavity of both species of shrews especially in the shoulder region. In heavy infections the larvae were found in the liver and other organs such as the wall of the stomach, oesophagus and intestine and in the thoracic cavity, sometimes on the surface of the lungs. When removed from the cysts the nematodes measure 15-19mm in length with a maximum diameter of 0.4mm. They appear green or yellow-green when encysted, but when removed from the cyst they

appear whitish with a green or yellow-green intestine.

Discussion

The L4 larvae found encysted in the body cavity of both *S.araneus* and *S.minutus* could be identified as belonging to the genus *Porrocaecum*. Due to superficial differences in pigmentation and size of the larvae it was thought possible that more than one species was present. The only reliable method of identification to species level would be to infect experimentally birds such as chickens or ducklings with larvae obtained from freshly killed shrews. After a suitable time interval the birds could be sacrificed and the adult worms (if they had developed) could be dissected out and identified. Such a procedure was beyond the scope of this project.

The shrew is acting as a paratenic host and development of the adult would only occur if the L4 larvae were ingested by a suitable definitive host. The definitive host in this case is therefore likely to be a predator of shrews such as the owl or another bird of prey. *Porrocaecum spirale* (Rudolphi 1795) Baylis 1920 has been found in the tawny owl, *Strix aluco* and the barn owl, *Tyto alba* in England (Hartwich 1959), so it seems likely that the nematodes found in the present study belong to this species.

Porrocaecum sp. has been recorded several times in this country in both *S.araneus* and *S.minutus*.

Family Heligmosomidae Cram, 1927.

Small nematodes, 1-2mm in length, belonging to the genus *Longistriata* were recovered from the intestine of both host species. They are identified by the eight longitudinal cuticular ridges. Under the SEM numerous transverse cuticular striations were also observed. The cuticle becomes dilated at the anterior end to form a cephalic vesicle. The males possess a relatively large copulatory bursa, the shape of which is diagnostic for the species. The length

of the spicules is approximately three and a half times the body width.

The taxonomy of this genus requires extensive revision which is beyond the scope of the present study. Specimens of *Longistriata* recovered were provisionally identified as follows: *L.didas* Thomas, 1953, *L.depressa* (Dujardin, 1845), *L.thomasi* (Thomas, 1953), *L.trus* Thomas, 1953.

Longistriata didas - Male worms of this species measure 1.2mm in length, with a maximum width of 47μm. The cephalic vesicle measures 41 x 28μm. The caudal bursa (Plate 3.6) measures 180x140μm. The length of the spicules ranges from 163 to 183μm and the gubernaculum is 35μm long.

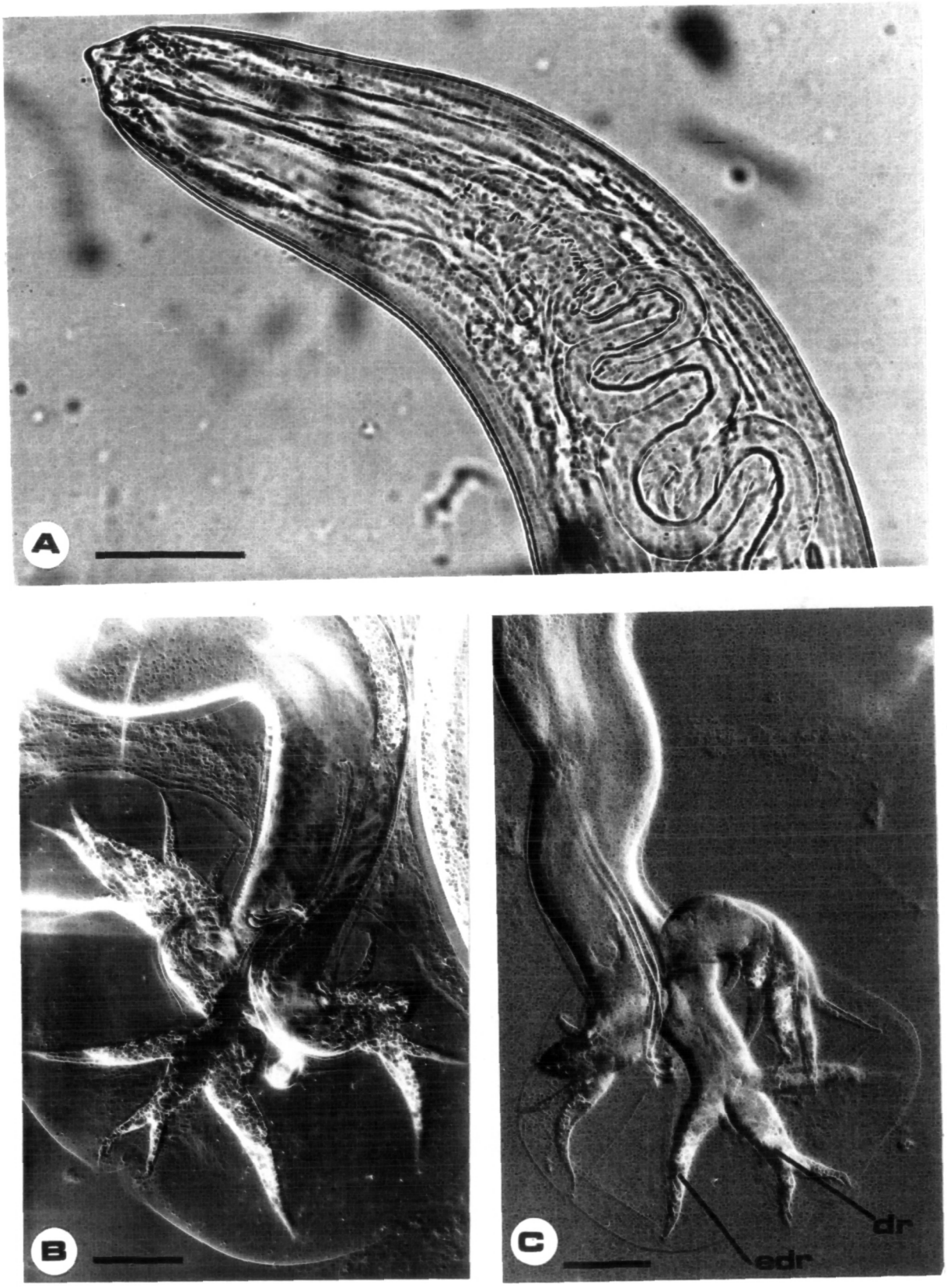
Females measure 1.5 to 1.8mm in length with a maximum width of 78μm. The cephalic vesicle measures 47 x 37μm and the oesophagus is 205-210μm in length. The cuticular ridges of the synlophe ended about 600μm from the posterior end of the worm. The size of the eggs was variable with a mean of 58 x 39μm.

L.depressa - The caudal bursa of the males (Figure 3.2) resembled the drawings made by Thomas (1953), Desportes & Chabaud (1961) and Vaucher & Durette-Desset (1973).

L.thomasi - Male specimens were recovered with a caudal bursa similar to that of *L.thomasi* described by Thomas (1953) and therefore assigned to this species.

L.trus - Males measure 1.5 - 1.8mm in length with a maximum diameter of 50μm. The cephalic vesicle measures 49x27μm and the oesophagus 185μm. The longitudinal cuticular ridges begin immediately behind the cephalic vesicle and end about 400μm from the end of the caudal bursa. The bursa measures 170x150μm and the spicules range from 171-185μm in length.

Apart from the females identified as *L.didas* three other types were found: (1) Nematodes with a very prominent swelling of the cuticle in the caudal region (Figure 3.2) resembled females assigned to *L.codrus* (=*L.depressa*) by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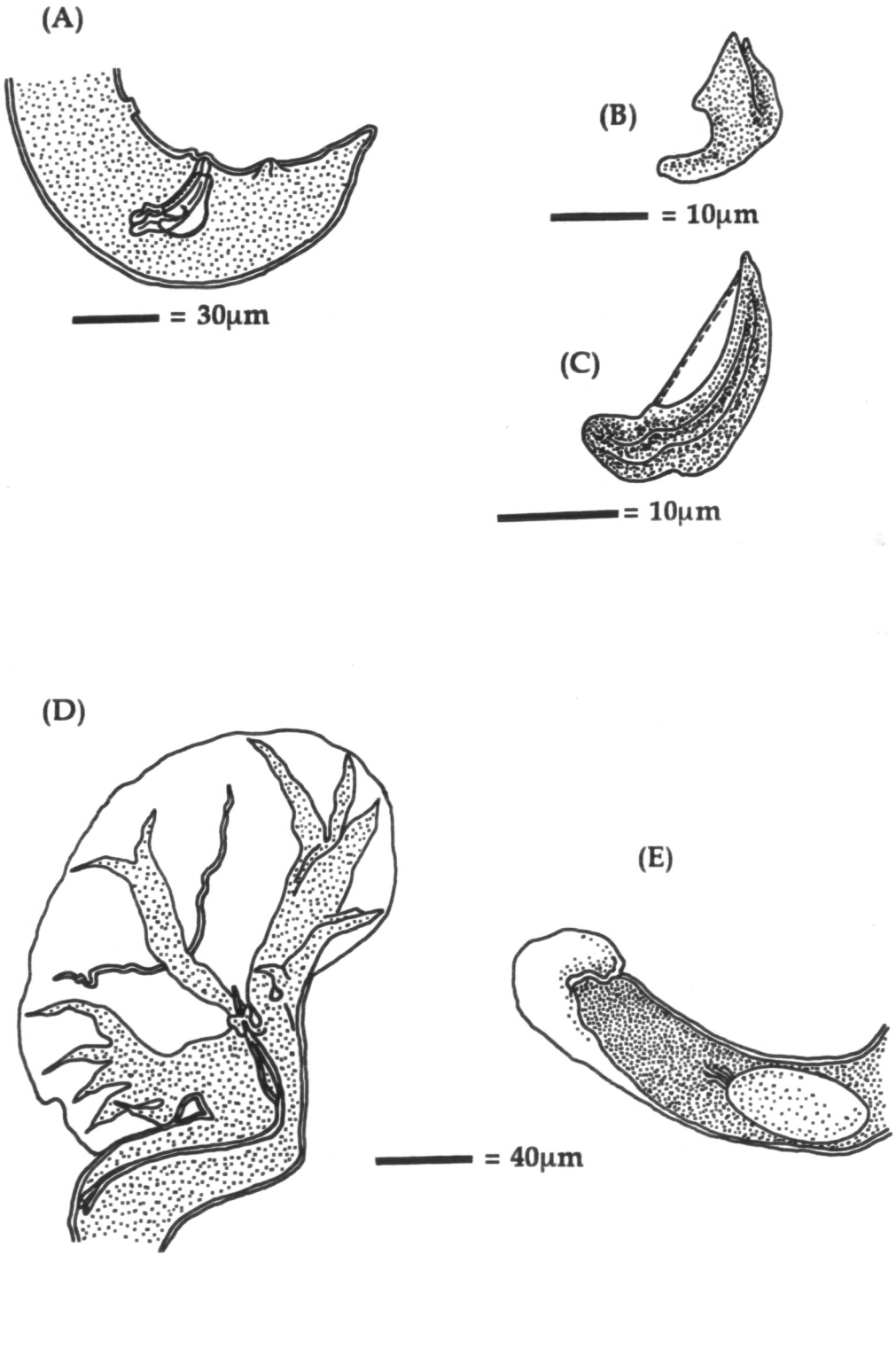

(A)
= 30µm
(B)
= 10µm
(C)
= 10µm
(D)
(E)
= 40µm

Thomas (1953) and were therefore assigned to the species *L.depressa*. Those recovered in the present study are 1.1-1.5mm in length with a maximum width of 50-58μm. The cephalic vesicle measures 44-64 x 24-37μm and the oesophagus 189-200μm. 4-5 eggs measuring 44-57 x 24-34μm are present in the uterus. The longitudinal ridges begin immediately behind the cephalic vesicle and terminate 385μm from the posterior end. The synlophe, examined in transverse section, is similar to that described by Vaucher & Durette-Desset (1973) for *L.depressa*. Some specimens appeared to possess a long, thin cuticular spike, about 15-20μm long, at the caudal end.

(2) Female nematodes were recovered which possess a distinct caudal bulge of the cuticle as well as a caudal spike. These resemble those of *L.thomasi* (=*L.depressa* Thomas 1953) and are therefore assigned to this species. The cephalic vesicle measures 63 x 42μm and the eggs 57-60 x 39-44μm.

(3) Female nematodes with a long thin cephalic vesicle and a prominent terminal spike (29-47μm long), but no caudal bulge were assigned to the species *L.trus*.

Discussion

There are comparatively few records of *Longistriata* spp. from *S.araneus* and *S.minutus* in the literature. This may, perhaps, be attributed to their small size making them easily overlooked. In general *Longistriata* spp. were less well described than other helminth species described in the present study, and this made their identification more difficult.

Longistriata didas was first described by Thomas (1953). Desportes & Chabaud (1961) recorded a nematode very similar to that of Thomas, but it differed in that the externo-dorsal rays of the caudal bursa branched at the mid-point of the dorsal ray, rather than at the proximal end of the ray as drawn by Thomas. Vaucher & Durette-Desset (1973) found similar specimens to those of Desportes & Chabaud (1961) and considered them to be a new species, *L.pseudodidas*. Since its description by Vaucher & Durette-Desset (1973), *L.pseudodidas* has been recorded by Mas Coma & Gallego (1975), Murai &

Mészáros (1984), Genov & Hadjinikilova (1984) and Haukisalmi (1989). *L.didas*, however, has not been recorded since it was found by Prokopic (1959).

In the present study there initially appeared to be two "*Longistriata didas* like" forms of male *Longistriata*, one of which was assigned to *L.didas* and the other to *L.pseudodidas* (Plate 3.6). However, as the study progressed, it became increasingly difficult to differentiate between the two forms as the externo-dorsal rays appeared to arise at varying points along the dorsal ray. The apparent position of the externo-dorsals varied with the orientation of the specimen.

The descriptions of the females of *L.didas* and *L.pseudodidas* in the literature are very similar and correspond to those found in the present study.

In the light of the above evidence it appears that the drawing of *L.didas* made by Thomas (1953) was either incorrect or unrepresentative and that the later work by Desportes & Chabaud (1961) and Vaucher & Durette-Desset (1973) constituted an improvement in the description of *L.didas* rather than a description of a new species. For this reason the names *L.didas* and *L.pseudodidas* have been synonymised by the present author.

Male specimens of *L.depressa* examined in the present study corresponded to those described by previous authors including Thomas (1953), Vaucher & Durette-Desset (1973) and Genov & Hadjinikilova (1984). The latter two authors produced similar descriptions of females of *L.depressa* which corresponded to the specimens assigned to *L.trus* in the present study, justifying their identifications by the similarity of the synlophe to that of the male of *L.depressa*. However, the synlophe of the females assigned by the present author to *L.depressa* was examined and found to be very similar to that described by the two authors mentioned above. The female specimens assigned to *L.depressa* in the present study were similar to those described by Thomas (1953).

Male specimens of *L.trus* in the present study were similar to those described by Thomas (1953) and Vaucher & Durette-Desset (1973). As already mentioned above female specimens identified in the present study as *L.trus* were similar to those described by Durette-Desset (1973) and Genov &

Hadjinikilova (1984) as *L.depressa*. Female specimens of *L.trus* have not previously been recorded.

A summary of the provisional conclusions reached as to the identity and taxonomy of the specimens of *Longistriata* in the present study are as follows:

(1) *L.didas* Thomas 1953 = *L.pseudodidas* Vaucher & Durette-Desset 1973; male drawn wrongly by Thomas.

(2) *L.thomasi* (Thomas 1953) Desportes & Chabaud 1961 = *L.depressa* (Dujardin 1845) Thomas 1953; male and female found in the present study corresponding to those found by Thomas (1953).

(3) *L.depressa* (Dujardin 1845) Desportes & Chabaud 1961 = *L.codrus* Thomas 1953. Male and female as drawn by Thomas; female not as drawn by Vaucher & Durette-Desset (1973) and Genov & Hadjinikilova (1984).

(4) *L.trus* Thomas 1953 was found in the present study. Male as described previously by Thomas (1953) and Vaucher & Durette-Desset (1973); female similar to that described by both Vaucher & Durette-Desset (1973) and Genov & Hadjinikilova (1984) as *L.depressa*.

Further work is required to clarify the taxonomy of the genus *Longistriata*.

Family Strongyloididae Chitwood & McIntosh, 1934

Specimens of the tiny nematode, *Parastrongyloides winchesi*, were found in the mucosa of the intestinal wall of both shrew species. The worms are similar in size to *Longistriata* sp., but could readily be distinguished by their lack of longitudinal ridges. The cuticle of *P.winchesi* has faint transverse striations only visible at high magnifications. In the male there is one pre-anal and two post-anal papillae, the spicules are shaped like a dagger and are enfolded by the gubernaculum which is shaped like a scoop (Figure 3.2). These features were

also described by Morgan (1928), but his drawings do not clearly demonstrate the form of the spicules. The specimens found in the present study were smaller than those of Morgan (1928) (Tables 3.22, 3.23) but apart from the size differences they did not differ in appearance. No difference was found between specimens recovered from *Sorex araneus* and those from *S.minutus*.

Table 3.22. Morphometric data on male *Parastrongyloides winchesi* for comparison with those obtained by Morgan, 1928.

Characteristic	No. examined	Range	mean	S.D.	After Morgan (1928)
Worm length	10	677-988	794	77.4	1000
Oesophagus length	8	275-351	319	26.1	460[1]
Maximum width	10	28-34	30	1.7	40
Width at ant. end	9	7-12	10	1.7	15
Width at spicules	9	24-29	27	1.6	40
Length of tail	9	30-38	35	2.3	40
Spicule length	9	28-32	29	1.1	40
Gubernaculum length	9	15-20	18	1.6	<spicules

(All measurements in μm.)

[1] 360 μm in the 'shrew'

Table 3.23. Morphometric data on female *P.winchesi* and a comparison with data obtained by Morgan, 1928.

Characteristic	No. examined	Range	mean	S.D.	After Morgan (1928)
Worm length	9	838-1188	1078	110	1200-1600*1
Oesophagus length	7	285-325	313	16.0	430-540*2
Maximum width	8	35-44	40	3.7	-
Width at ant. end	7	10-13	11	1.2	10
Width at anus	4	13-17	15	1.5	20
Width at vulva	5	35-44	40	3.8	35
Length of tail	5	32-45	37	5.4	50
No. of eggs	8	0-16	7	4.7	<10*3
Egg length	20	44-65	54	4.6	40
Egg width	20	27-42	32	5.0	20
Distance from vulva to posterior end	6	349-385	374	13.7	500

(All measurements in μm.)

*1 Morgan also observed a larger group of females c.2200 μm in length.

*2 390 μm in the "shrew".

*3 >40 in the larger group of females.

Discussion

Parastrongyloides winchesi was discovered by Morgan (1928) in the mole (*Talpa europaea* L.). He also found the parasite in a 'shrew' although he does not state which species of shrew. He found that the specimens from the shrew were slightly smaller than those recovered from the mole (differing particularly in the length of the oesophagus), but he did not consider the differences to be taxonomically significant. Cameron and Parnell (1933) recorded *'Strongyloides sp.'* in *S.minutus* in Scotland. Their specimen was too degenerate to identify, but it is likely to have been *P.winchesi*. Joyeux & Baer (1937) recorded *P.winchesi* in

Crocidura russula. They stated that their specimens were slightly smaller than those found by Morgan in the mole, but did not give any further information.

There were no differences in physical characteristics of the parasites found by Morgan (1928) in the mole and those found by the present author in *S.araneus* and *S.minutus* except for size differences. Almost every measurement made was smaller in the nematodes from the shrew than those from the mole (Tables 3.22, 3.23). It may be that the specimens recovered from *S.araneus* and *S.minutus* are a separate species from that found in the mole or that the same species develops differently in moles and shrews, but a direct comparison of specimens from the mole and shrew would be required in order to establish this.

Family Trichuridae

Sub-family Capillariinae Nevue-Lemaire, 1936

Four Capillariinid species were found in the present study, namely *Eucoleus kutori, E.oesophagicola* and *Liniscus incrassatus* which were found in both shrew species, and *Calodium cholidicola* which was found only once in a common shrew at "Huntersdale".

The single specimen of *Calodium cholidicola* recovered is a female found tightly coiled in the parenchyma of the liver. It measures 19mm in length with a maximum width of 290μm. The oesophagus is short when compared with the overall worm length, being only 2.640mm long. The worm is covered with subcuticular papillae approximately 3μm in diameter.

Specimens of *E.oesophagicola* were found in the mucous membrane of the oesophagus in a flat spiral with many coils. They were not found in the stomach and thus occupied a different niche from *E.kutori*. The male has a rudimentary bursa which is almost invisible and the spicule sheath is armed with large spines. The female contains a large number of eggs, each having two polar plugs, characteristic of the Trichuridae. Morphometric data (Tables 3.24, 3.25) identify the specimens found in the present study as the same species found by Romashov (1983). The distance from the vulva to the posterior end (10519-12527μm) was not measured by the latter author.

Table 3.24. Morphometric data on male *Eucoleus oesophagicola* and a comparison with data obtained by Romashov, 1983.

Characteristic	No. examined	Range	mean	S.D.	After Romashov (1983)
Worm length	5	6673-10058	7701	1371	5800-9910
Oesophagus length	5	2284-3168	2724	318	1960-2940
Body length	5	3856-6890	4977	1170	3680-5250
Oesophagus: Body	5	1:1.37-1:2.18	1:1.83	-	-
Maximum width	5	59-91	69	12.9	65-97
Width at post. end	5	30-35	34	2.0	29-32
Spicule length	1	-	c.240	-	160-220
Spicule sheath length	5	93-176	c.140	-	140-190

(All measurements in μm.)

Table 3.25. Morphometric data on female *Eucoleus oesophagicola* and a comparison with data obtained by Romashov, 1983.

Characteristic	No. examined	Range	mean	S.D.	After Romashov (1983)
Worm length	3	14032-16236	15431	1216	8650-14790
Oesophagus length	2	3450-3487	3469	-	2440-3400
Body length	2	10545-12575	11560	-	6480-11650
Oesophagus: Body	2	1:3.02-1:3.65	1:3.34	-	-
Maximum width	4	82-106	95	11.7	83-120
Width at post. end	4	34-54	42	11.9	29-39
Width at vulva	3	80-117	89	24.7	65-90
Egg length	14	64-82	72	4.7	73-84
Egg width	16	27-34	30	2.3	30-39
Distance from vulva to posterior end	2	10519-12527	11500	-	-

(All measurements in μm.)

Specimens of *Eucoleus kutori* are found in the lumen of the stomach of *Sorex araneus* and *S.minutus*. In addition to occupying a different niche to *E.oesophagicola*, *E.kutori* is about one third of the length, has a relatively longer oesophagus, longer spicules and smaller eggs (Tables 3.26, 3.27).

Liniscus incrassatus, found in the bladder, is characterised by the long, very thin oesophageal region (Plate 3.2, Tables 3.28, 3.29). The spicule and spicule sheath are not easily seen; the spicule sheath does not possess spines. Morphometric data on these nematodes are given in Tables 3.28 and 3.29.

Table 3.26. Morphometric data on male *Eucoleus kutori* (present study)

Characteristic	No. examined	Range	mean	S.D.
Worm length	3	4422-4554	4477	68.7
Oesophagus length	3	1907-2277	2064	191.3
Body length	3	2277-2515	2413	122.6
Oesophagus:body length	2	1:1.00-1:1.32	1:1.18	-
Maximum width	5	47-49	47	0.8
Spicule length	4	342-384	368	18.7
Spicule sheath length	4	318-436	384	49.4

(All measurements in μm.)

Table 3.27. Morphometric data on female *Eucoleus kutori* (present study)

Characteristic	No. examined	Range	mean	S.D.
Worm length	4	9656-10606	10144	393.4
Oesophagus length	4	3478-4059	3800	261.9
Body length	4	5953-6600	6344	335.6
Oesophagus:body length	4	1:1.52-1:1.90	1:1.68	-
Maximum width	5	84-102	93	6.7
Width at vulva	4	67-74	69	3.2
Egg length	14	57-64	60	2.3
Egg width	14	22-27	24	1.2
Distance from vulva to posterior end	4	5953-6590	6319	316.3

(All measurements in μm.)

Table 3.28. Morphometric data on male *Liniscus incrassatus* (present study)

Characteristic	No. examined	Range	mean	S.D.
Worm length	3	6758-11088	8889	2166
Oesophagus length	3	3524-4673	4107	575
Body length	3	3234-6415	4782	1592
Oesophagus:body	3	1:1.09-1:1.37	1:1.14	-
Maximum width	4	67-94	78	11.6
Width at ant. end	4	8-10	9	1.0
Width at post. end	4	20-27	23	3.0
Spicule length	5	776-938	860	71.2
Spicule sheath length	3	863-1585	1194	364.9

(All measurements in μm.)

Table 3.29. Morphometric data on female *Liniscus incrassatus* (present study)

Characteristic	No. examined	Range	mean	S.D.
Worm length	3	10171-13097	11822	1499
Oesophagus length	3	5610-6514	6061	452
Body length	3	4561-6583	5760	1062
Oesophagus:body	3	1:0.81-1:1.01	1:0.94	-
Maximum width	4	96-184	142	36.3
Width at vulva	3	69-100	81	16.9
Width at ant. end	4	7-9	8	1.1
width at post. end	4	30-39	34	2.7
Egg length	10	50-60	56	4.1
Egg width	10	22-27	26	1.7
Distance from vulva to posterior end	3	4231-6372	5507	1128

(All measurements in μm.)

Discussion

The taxonomy of the Capillariinae was revised by Moravec (1982). Three of the four capillariinid species found in the present investigation could be classified to generic level according to key produced by the above author. The fourth species *Calodium cholidicola* was only found on one occasion and since the specimen was female it could not be identified using Moravec's key which relies heavily on characteristics of the male. However, characteristics of the female enabled successful identification to be achieved. The specimen had similar dimensions to those recorded by Soltys (1952) and also possessed the subcuticular papillae observed by this author. The oesophageal region was very short, a diagnostic feature according to Wakelin (1968). The location in the host, tightly coiled in the

parenchyma of the liver, is also in agreement with the results obtained by Soltys (1952).

This is the second record of *Calodium cholidicola* from a British shrew, the first record being by Wakelin (1968).

Eucoleus oesophagicola was first described (although not in great detail) from Poland by Soltys (1952). A more comprehensive description was produced recently by Romashov (1983). This redescription compares favourably with morphological data obtained in the present investigation (Tables 3.24 and 3.25).

The first British record of *E.oesophagicola* was by Lewis (1964) in *S.araneus*. Lewis (1987) also found this parasite in *S.minutus*.

Eucoleus kutori has only been recorded once before in Britain, in the intestine of a common shrew from England (Wakelin, 1968). The specimens found in the present investigation were recovered from the stomach. Thomas (1953) and Sharpe (1964) recorded the presence of '*Capillaria exigua* Dujardin 1845' in the stomach of *S.araneus*. *Capillaria exigua* (now referred to as *Eucoleus exigua*) is clearly distinct from *Eucoleus kutori*, but is superficially similar. Thus it appears that the specimens found by Thomas were wrongly identified and that this error was also made by Sharpe who used Thomas' paper for reference; neither author gave a description of the parasite.

'*Capillaria petrowi*' was reported by Prokopic (1959) in Czechoslovakia, this parasite is similar to *E.kutori* so this may be another case of incorrect identification; again no description is given.

E.kutori has not been reported previously in *S.minutus* which is therefore a new host record.

The taxonomy of *Liniscus incrassatus* has been the subject of much debate in the literature as discussed by Mas Coma & Gallego (1975). In particular the names *Capillaria incrassata* and *Capillaria capillaris* have been used indiscriminately without reasons being given for the choice. At present it appears sensible to consider all species of nematodes from the bladder of *S.araneus* and *S.minutus* as *Liniscus incrassatus* until a satisfactory investigation has been carried out.

Soltys (1952, 1954) recorded two Capillariinid species in the bladder of *S.araneus* and *S.minutus*: *Capillaria incrassata* and *C.capillaris* (which he called

'C.urinicola' in 1952). The morphological data obtained in the current investigation are closer to those for *C.incrassata* (Soltys, 1952), but Soltys does not provide very comprehensive descriptions of the species which he found.

Liniscus incrassatus was first recorded in Britain (under the synonym *Capillaria incrassata*) by Baylis (1928) in *S.araneus*. *C.incrassata* was also recorded in *S.araneus* by James (1954) and Lewis (1964, 1968, 1987). Lewis (1964) recorded *C.incrassata* in *S.minutus*.

L.incrassatus was present in both *S.araneus* and *S.minutus* in the current investigation.

Unidentified Larval Nematodes

Several specimens of both *S.araneus* and *S.minutus* were infected with very small larval nematodes (nematode larva, a) located in the liver and sometimes in the intestinal or oesophagus wall. The cysts containing the larvae are 500-660μm in diameter and contain 1-5 larvae. The larvae are 400-800μm in length with a maximum width of 20-30μm, the diameter at the anterior end is 10μm and the distance from the anus to the posterior end is 84μm. It was not possible to identify the nematodes due to their lack of taxonomically significant characteristics.

Larger nematode larvae (nematode larva, b) 3.040mm in length with a maximum diameter of 112μm were found encysted in the liver of a few specimens of *S.araneus* (Plate 3.6).

An up-to-date list of the species of nematodes recorded in *Sorex araneus* and *S.minutus* in Europe has been compiled and is given below. All known synonyms of species found in the present study are also listed.

A key to the adult nematodes found in *S.araneus* and *S.minutus* in Britain is also given below.

Nematodes (with synonyms) found in European *S.araneus* and *S.minutus* with Records of Species Found in the Present Study.

Family Sobilophymatidae

Sobilophyme soricis Baylis & King 1932.

Family Trichuridae

Sub-family Capillariinae (Nevue-Lemaire 1936).

Calodium cholidicola (Soltys 1952) Moravec 1982. *

Synonyms and records:

Capillaria cholidicola (Soltys 1952) Wakelin 1968.

Capillaria hepatica sensu Stunkard et al. 1975 ?

Hepaticola cholidicola (Soltys 1952) Prokopic 1959; Andreiko 1973.

Calodium splenaecum (Dujardin 1843) Dujardin 1845.

Records:

Calodium splenaceum (Dujardin 1845) Molin 1861; Moravec 1982.

Synonyms and records:

Capillaria splenaeca (Dujardin 1843) Soltys 1952.

Capillaria splenaceum (Diesing 1851) Travassos 1915.

Trichosoma splenaecea Diesing 1851.

Trichosoma splenaecum Dujardin 1843.

Trichosoma splenaecum (Dujardin 1843) Stossich 1890.

Eucoleus kutori (Ruchladiew 1946) n. comb. *

Synonyms and records:
Capillaria exigua sensu Thomas 1953, Sharpe 1964 ?
Capillaria kutori (Ruchladiew 1946) Soltys 1954; Prokopic 1959; Wakelin 1968; Andreiko 1973; Mas Coma & Gallego 1975; Murai & Meszaros 1984; Haukisalmi 1989.
Capillaria petrowi Ruchladiew 1949 sensu Prokopic 1959 ?
Capillaria rauschi Read 1949.
Capillaria ventricola Soltys 1952; Stammer 1955.

Eucoleus oesophagicola (Soltys 1952) Skrjabin & Shikhobalova 1954. *

Synonyms and records:
Capillaria blarinae Ogren 1953
Capillaria oesophagicola Soltys 1952; Soltys 1954; Stammer 1955; Lewis 1964, 1968, 1987.
Thominx blarinae (Ogren 1953) Skrjabin & Schikhobalova 1954; Prokopic 1959.
Thominx oesophagicola (Soltys 1952) Murai & Meszaros 1984.

Liniscus incrassatus Diesing 1851 *

Synonyms and records:
Capillaria capillaris (Linstow 1882) Stiles & Stanley 1932; Soltys 1954.
Capillaria incrassata (Diesing 1851) Soltys 1952; James 1954; Prokopic 1959; Lewis 1964, 1968; Andreiko 1973; Murai & Meszaros 1984; Lewis 1987.
Capillaria incrassata (Diesing 1854) Stammer 1955; Mas Coma & Gallego 1975.
Capillaria linstowi Travassos 1914.

Capillaria sunci Chen 1937.
Capillaria urinicola Soltys 1952.
Liniscus exilis Dujardin 1845.
Trichosoma incrassata Diesing 1851.
Trichosoma incrassatum (Diesing 1851) Stossich 1890.

Sub-family Trichurinae

Trichuris busuluk (Pologentsev 1935).
Syn: *Trichocephalus busuluk* Pologentsev 1935.

Family Ascarididae Baird 1853.
Genus *Porrocaecum* Railliet & Henry 1912. *
Records:
Pologentsev 1935; Soltys 1952, 1954; Thomas 1953; Stammer 1955; Prokopic 1959; Sharpe 1964; Lewis 1964, 1968, 1987; Buckner 1969; Andreiko 1973; Mas Coma & Gallego 1975; Erkinaro & Heikura 1977; Grainger & Fairley 1978; Churchfield 1979; Murai & Meszaros 1984.

Family Acuariidae Seurat 1913.

Stammerinema soricis (Tiner 1951) Osche 1955. *

Records:
Osche 1955; Prokopic 1959; Churchfield 1979.

Synonyms and records:
Dispharynx soricis Tiner 1951. (In *Sorex obscurus alascensis*).
Synhimantus rhopalocephalus Soltys 1952 (*S.araneus*); Soltys 1954 (*S.minutus*).

Paracuaria soricis Jančev 1972.

Family Angiostrongylidae

Stefanskostrongylus soricis (Soltys 1954) Drozdz 1970. *

Records:

Mas Coma & Gallego 1975; Biocca et al. 1976; Mas Coma 1977.

Synonyms and records:

Angiostrongylus soricis Soltys 1954; Prokopic 1959.

Family Skrjabingylidae

Paracrenosoma skrjabini (Pologentsev 1935) Yun & Kontrimavichus 1963.

Syn: *Crenosoma skrjabini* Pologentsev 1935.

Family Strongyloididae Chitwood & McIntosh 1934.

Parastrongyloides winchesi Morgan 1928 *

Records:

Morgan 1928; Cameron & Parnell (1933)?; Thomas (1953); Stammer 1955; Prokopic (1959); Andreiko 1973; Mas Coma & Gallego (1975); Murai & Meszaros (1984); Haukisalmi (1989).

Family Heligmosomidae Cram 1927.

Longistriata baeri Vaucher & Durette-Desset 1973.

(Recorded by San Martin-Duran et al. 1987)

Longistriata didas Thomas 1953 *

Records:
Prokopic 1959; Desportes & Chabaud 1961.

Synonyms and records:
Longistriata pseudodidas (Desportes & Chabaud 1961) Vaucher & Durette-Desset 1973; Mas Coma & Gallego 1975; Murai & Meszaros 1984; Genov & Hadjinikilova 1984; Haukisalmi 1989.

Longistriata depressa (Dujardin 1845) Desportes & Chabaud 1961. *

Records:
Desportes & Chabaud 1961; Vaucher & Durette-Desset 1973; Murai & Meszaros 1983; Genov & Hadjinikilova 1984; San Martin-Duran et al. 1987; Haukisalmi 1989.

Synonyms and records:
Heligmosomum depressum Railliet & Henry 1909.
Longistriata codrus Thomas 1953; Stammer 1955; Prokopic 1959; Andreiko 1973.
Longistriata ljamkini Eltichev 1975.
Strongylus depressus Dujardin 1845.
Viannaia depressa Travassos 1918.

Longistriata thomasi (Thomas 1953) Desportes & Chabaud 1961

Synonym:
Longistriata depressa (Dujardin 1845) sensu Thomas 1953; Prokopic 1959?

Longistriata trus Thomas 1953.

Records:

Stammer 1955; Andreiko 1973; Vaucher & Durette-Desset 1973.

* = found in the present study

? = the most likely identity of the helminth recorded by the author.

Key to the Nematode Parasites of *Sorex araneus* and *S.minutus* in Britain.

1.	Long, thin oesophageal region, usually as long as rest of the body; eggs with a polar plug at each end	Capillariinae	2
	Comparatively short oesophageal region; eggs without polar plugs		5
2.	Located in parenchyma of liver; body covered in subcuticular papillae	*Calodium cholidicola*	
	Not found in liver; no subcuticular papillae		3
3.	Located in bladder; spicules > 700μm	*Liniscus incrassatus*	
	Found in alimentary canal; spicules < 400μm		4
4.	Located in stomach; eggs 57-64μm long	*Eucoleus kutori*	
	Found in oesophagus; eggs 64-87μm long	*Eucoleus oesophagicola*	
5.	Less than 2mm long; found in intestine		6
	More than 2mm long; not found in intestine		7
6.	Synlophe consists of eight longitudinal cuticular ridges; spicules approximately 3.5 body width	*Longistriata spp.*	
	No synlophe; spicules shorter than body width	*Parastrongyloides winchesi*	
7.	Located in stomach; head bears four thick cuticular ridges known as cordons	*Stammerinema soricis*	
	Found in lungs; no cordons	*Stefanskostrongylus soricis*	

Acanthocephalans

Two species of acanthocephala were recorded in the present study. A single adult female specimen of *Prosorhynchus* sp. was recovered from a common shrew in the grounds of "Alderhurst". The proboscis of this specimen was not fully everted so it was not possible to identify it to species. The shrew was not considered to be the usual definitive host of this parasite; this role probably being fulfilled by a passerine bird.

Acanthella stages of *Gordiorhynchus aluconis* were found in the body cavity of both host species, often attached to the gut mesenteries, more rarely in the parenchyma of the liver. They resemble small white seeds, less than 2mm long. They could be excysted by placing them in distilled water revealing the spiny proboscis, characteristic of acanthocephala. The numbers of rows of spines and hooks and the number in each row were counted using SEM. These data along with other measurements made using light microscopy are shown in Table 3.30. The shape of the spines changes along the length of the proboscis (Plate 3.7. "Spines" 1-3 are hooklike, "spines" 4-7 are thick spines and those remaining are thin spines.

Ewald (personal communication) examined adult specimens of *G.aluconis* from the tawny owl *Strix aluco* and found that male specimens had 29-33 rows of 14-17 hooks and spines and females had 27-31 rows of 15-17. The specimens examined in the present study had 28-30 rows of 13-17 hooks and spines and could therefore be identified as *G.aluconis*. The present specimens were also very similar to specimens of *G.aluconis* obtained from *S.aluco* by the present author and examined using SEM.

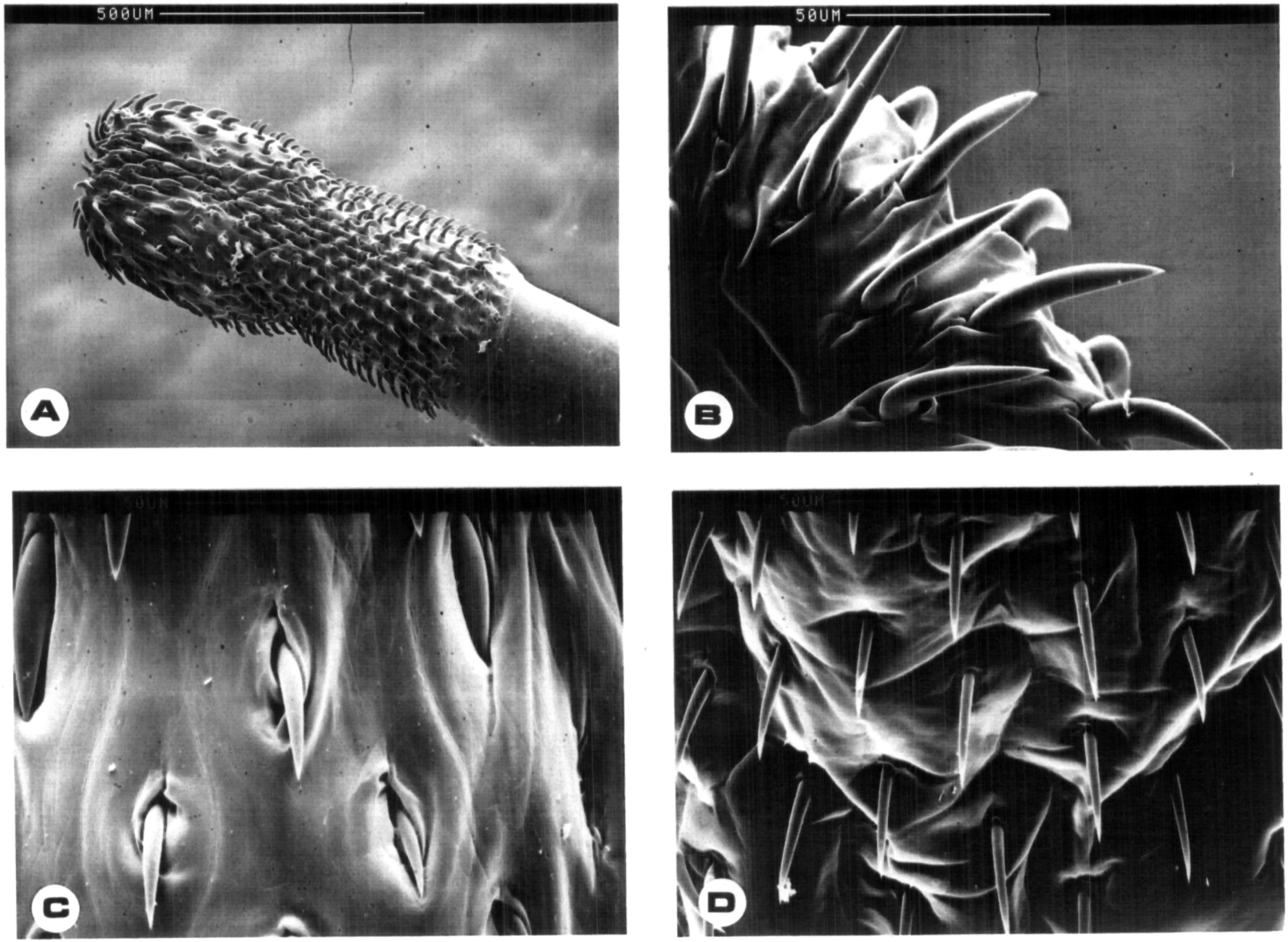

500UM
A
50UM
B
C
D

Table 3.30. Morphometric data on the acanthella stages of *Gordiorhynchus aluconis* (all measurements in mm).

Characteristic	No. examined	Range	mean	S.D.
No. of rows of "spines"	10	28-30	29	0.88
No. per row	10	13-17	15	0.54
Encysted length	10	1.20-1.63	1.43	0.15
Excysted length	10	3.35-5.00	3.99	0.48
Width	10	0.54-0.81	0.66	0.08
Tail length	10	0.93-2.30	1.39	0.38
Proboscis length	8	0.94-1.45	1.23	0.19
Length of spiny part of proboscis	10	0.70-1.05	0.87	0.11

Discussion

The presence of acanthocephalan larvae in *S.araneus* and *S.minutus* has been recorded by a number of authors (Baylis, 1928; Soltys, 1952, 1954; Prokopic, 1959; Lewis, 1964, 1968; Canning et al., 1973; Grainger & Fairley, 1978; Murai & Meszaros, 1984; Ewald et al., 1991). Most of these authors have identified their species as either *Centrorhynchus* (=*Gordiorhynchus*) *aluconis* or *C.buteonis*. However Soltys (1952, 1954) is the only one to have given a description and his descriptions were not sufficient to identify the species present. Data on adult *G.aluconis* obtained from J.A.Ewald (personal communication) and an examination of mature specimens of *G.aluconis* enabled the present author to establish firmly that the acanthella stages found in *S.araneus* and *S.minutus* during the current investigation belong to this species.

The name *Gordiorhynchus* Meyer 1931 is used rather than *Centrorhynchus* Luhe 1911 because *Centrorhynchus* was used by Waldheim in 1829 for a genus of

Coleoptera. *Centrorhynchus* was not used as a generic name for Acanthocephala until 1911. The invalidity of the name *Centrorhynchus* for acanthocephalans was discussed by Dollfus & Golvan (1957).

Summary

Twenty-nine species of helminths comprising ten species of cestodes, three digeneans, fourteen nematodes and two acanthocephalans, have been recovered from British specimens of *S.araneus* and *S.minutus* and their taxonomy and descriptions revised where necessary. This is a significant expansion of the original British list. Complete lists of helminth parasites present in European specimens of the two host species have been compiled. Apart from the helminth species described above, the only other helminth species known to have been found in British *Sorex spp.* is the nematode, *Sobilophyme soricis* Baylis & King 1932 which has been recorded twice from Scotland. Several species recorded in mainland Europe have not been recorded in Britain. This may be due to the geographical isolation of Britain from the rest of Europe and also to the limited diversity of habitats examined. The species composition of the helminth fauna of shrews is known to vary according to habitat type (Prokopic 1959, Chapter 6 of the present study), for example *Opisthioglyphe sobolevi* was recorded only at the wetland site at Dungeness.

Chapter 4
Faecal Analysis as a Technique for Monitoring Egg Output of Helminth Parasites in *Sorex araneus* and *S.minutus*.

Introduction

The most reliable method of determining the prevalence and intensity of helminths in a host population is to cull a sufficiently large sample to be representative and to carry out a thorough post mortem of each host. However, this obviously has an undesirable affect on the population being studied. In the case of shrews, where population densities are not especially high, the removal of a sufficiently large sample to be representative of the population as a whole will tend to alter drastically the population structure. This will be exacerbated if successive samples are taken, as in the present study, in order to investigate seasonal changes in helminth infections.

To eliminate the deleterious effect of removal trapping, shrews from the Silwood Park population were not culled and were solely used to provide faecal samples, which were then examined for the presence of helminth eggs.

In addition to the minimal disturbance of the shrew population, the other advantages of faecal analysis are that the occurrence of helminth infections can be followed over a period of time and that faecal analysis may be carried out more rapidly than a thorough dissection.

The disadvantages of relying solely on faecal analysis as an indication of the prevalence and intensity of helminth infection are as follows: parasitic larvae are not detected - in the present study this would include the L4 larvae of *Porrocaecum sp.* and the acanthella stages of *Gordiorhynchus aluconis* which may be present in large numbers and presumably therefore have an appreciable pathological effect; male nematodes will also be undetected, as will immature parasites which may be highly pathogenic (M.A.F.F., 1986). A single sample may be unrepresentative and the number of eggs counted may not be a good indicator of the number of worms present in the host for reasons discussed below. Another problem associated with faecal analysis is the identification of the species of parasite by which the eggs were produced; nematode eggs are especially difficult to distinguish.

Several techniques involving the analysis of faecal samples have previously been employed for the purpose of monitoring helminth infections in mammals.

Such techniques have been used in agriculture for the enumeration of the eggs of helminths, usually nematodes, in sheep, cattle and pigs (M.A.F.F., 1986).

Faecal analysis techniques have usually been developed in the field of medical parasitology e.g. using faeces of humans infected with the hookworm, *Anclystoma duodenale*, (Stoll, 1923, Stoll & Hausheer, 1926, Lane, 1924, 1925) or using the faeces of mice infected with the nematode, *Heligmosomoides polygyrus* (Dunn & Keymer, 1986).

There are references in the literature to faecal analysis being used for various other host-parasite systems, e.g. *Ascaris lumbricoides* (Nematoda) in man (Sinniah, 1982; Chai et al., 1981); *Schistosoma mansoni* (Digenea) in man (Katz et al., 1972); *Hymenolepis diminuta* (Cestoda) in the rat (Hesselberg & Andreassen, 1975), but the only record of faecal analysis being used to look for helminth eggs in shrews is by Bock (1982). Bock demonstrated that shrews had been successfully infected with the digenean *Opisthioglyphe locellus* by recovering eggs in the faeces. Hence, prior to the present study, quantitative techniques had not been used to determine the numbers of helminth eggs present in samples of shrew faeces. It was therefore necessary to develop a suitable technique to identify and count the eggs of a range of helminth species found in *S.araneus* and *S.minutus*.

There are three aspects to be considered in the development of a technique for estimation of the worm burden from faecal egg counts:

(a) Description and measurement of the eggs of the parasites for identification from a sample of faeces.

(b) Development of a Technique to Measure E.P.G.

Techniques which have been used for faecal analysis can be divided into three categories: faecal smears, flotation techniques and dilution techniques.

Faecal smears were originally used to demonstrate the presence or absence of helminth eggs (M.A.F.F., 1986), but Beaver (1949) described a technique whereby faecal smears could be used to quantitatively estimate the number of eggs present. A fleck of faeces was taken at random from a stool sample and stirred into a drop of water on a microscope slide. By progressively adding faeces, a smear of standard turbidity (photoelectric measurement of density) was produced. The number of eggs present under a 22x22mm coverslip was then

counted. Since the turbidity of the sample was directly related to the amount of faeces in a set volume (i.e. the density) and the volume taken was constant, the amount of faeces counted was the same for each smear. Beaver found by comparison with other techniques that his method was reasonably accurate.

The above method was not considered practical for use in the present study because of the difficulties in calibration of the turbidity of the smear and the small quantity of shrew faeces per sample. The quantity was such that the whole sample would have been needed to make the initial smear, leaving no further faeces to be used to alter the turbidity.

Flotation techniques work on the principle that the density of helminth eggs is less than that of the rest of the faeces, so that if the faeces are mixed with a solution of a greater specific gravity than that of the eggs, they will float to the surface, while the debris will sink to the bottom of the counting chamber. The solution used for the flotation will depend on the eggs which the investigator wishes to detect. A 50% saturated solution of sodium chloride has a specific gravity (S.G.) of 1.125 and may be used to detect trichostrongylid and strongylid nematode eggs. A 100% saturated solution of sodium chloride (S.G. 1.204) is adequate for the detection of most cestode eggs, but a saturated solution of zinc sulphate (S.G. 1.364) is required to detect eggs of digeneans such as the liver fluke, *Fasciola hepatica* (M.A.F.F., 1986). Saturated sucrose solution (S.G. 1.286) has been successfully used by Jones & Tan (1971) to detect eggs of the cestode, *Hymenolepis microstoma*, in mouse faeces; Hesselberg and Andreassen (1975) used a 50% saturated sucrose solution to detect the eggs of *H.diminuta* in rat faeces.

The most commonly used flotation technique is the McMaster technique which employs a special slide known as a McMaster slide (M.A.F.F., 1986) which allows the eggs present in a known volume of solution to float to the top of the counting chamber allowing them to be easily counted. Dunn & Keymer (1986) have shown that for *Heligmosomoides polygyrus* (Nematoda) in mouse faeces there is an optimum flotation time of 30 minutes and an optimum sample dilution of 15ml of 50% saturated sodium chloride solution to one gramme of faeces.

A modified McMaster technique is described in the materials and methods section of this chapter.

Lane (1924) developed an alternative flotation technique known as "direct centrifugal flotation" which he considered was able to detect all the hookworm eggs present in a human faecal sample. In this technique eggs were brought to the surface of a centrifuge tube filled to the brim with a saturated sodium chloride solution by centrifuging at 1000 revolutions per minute (rpm) for one minute. The eggs adhered to a coverslip which had been placed on top of the tube and the eggs could be removed along with the coverslip.

A modified direct centrifugal flotation technique is described in the materials and methods section of this chapter.

One problem associated with flotation techniques is deformation of the eggs caused by the salt solution. Stoll & Hausheer (1926) found that hookworm eggs were deformed by saturated sodium chloride and Dunn & Keymer (1986) suggested that deformation of *H.polygyrus* eggs by a 50% saturated solution of sodium chloride was responsible for a decrease in number of eggs counted if the flotation time was increased above 30 minutes.

Dilution techniques are based on a technique developed by Stoll (1923). A known mass of faeces is diluted to a known volume of solution. Subsamples (of known volume) of the solution are pipetted onto a microscope slide and the number of eggs counted. A modified Stoll technique is described in the materials and methods section of this chapter.

(c) Estimation of the number of worms established in the host using faecal egg count data.

There are several problems in reliably estimating the worm burden from measurements of E.P.G. some of them are associated with the biology of the host and nature of the faeces while others are associated with the biology of the helminth parasites. The small quantity of faeces produced by the shrew means that a small error made in the weighing procedure will have a more marked effect than it would if larger samples of faeces were produced. The faeces are also very susceptible to drying out, causing a decrease in mass.

Hopkins (1985) found that daily faecal output varied by a multiple of two or three in the same individual mice. The number of eggs per gramme of faeces is therefore not necessarily proportional to the number of eggs produced per day.

Problems caused by the biology of the parasites include the irregular release of eggs into the intestine; eggs of cestodes are released in packets since gravid proglottids containing eggs, rather than the individual eggs themselves are released (Hall et al., 1981). Circadian rhythms of egg release have been demonstrated in several nematodes: *Syphacia muris* in the mouse (Van der Gulden, 1967; Lewis & D'Silva, 1980), *Heligmosomoides polygyrus* in the mouse (Lewis & Shava, 1977; Kerboeuf & Lewis, 1987), *Syphacia obvelata* in the rat and *Enterobius vermicularis* in man (Lewis & Shava, 1977). Circadian rhythms in egg output by cestodes have not been demonstrated, but Hopkins (1970) and Bailey (1971) demonstrated periodicity in the movement of *Hymenolepis diminuta* in the rat.

The fecundity of individual worms has been shown to decrease as the worm burden increases as demonstrated by Jones & Tan (1971) in *H.diminuta* and Kerboeuf (1985) in *Heligmosomoides polygyrus*. Another factor influencing fecundity is the age of the worms (Chai et al., 1981).

Attempts to estimate prevalences and intensities of helminth parasites using faecal egg counts are therefore beset with problems. However, the advantages of faecal analysis techniques - minimal disturbance of the host population and ability to follow temporal changes in infection of individual hosts - make such an approach worthy of consideration. This chapter describes the development of a technique for the qualitative and quantitative determination of helminth eggs in the faeces of shrews and its use to investigate the circadian rhythms of egg output and the relationship between E.P.G. and worm burden. The use of this technique to investigate seasonal variation in helminth infections of shrews will be considered in Chapter 6.

Materials and Methods

Identification of Eggs

In order to be able to identify the eggs found in the faecal samples mature parasites (females in the case of nematodes) of each species found in the shrew were examined and their eggs photographed, drawn and measured using a microscope fitted with an eyepiece graticule. Sufficient helminth material for this purpose was available from the autopsies of shrews from Windsor Great Park.

Collection of Samples

Most of the faecal samples were collected from Longworth traps in the field (Chapter 2), but samples were also collected in the laboratory using the following procedure:

The shrew was placed in a large sandwich box or gallon ice cream container along with non-absorbent cotton wool bedding and food (pupae of *Calliphora sp.*) and water in small glass pots. After four hours the shrew was transferred to a similar container and the faeces which had been produced were carefully collected using fine forceps and placed in a labelled specimen tube. This procedure was repeated for a total of 28 hours to investigate any diurnal variation in egg output. The shrews were kept under natural lighting conditions.

Two faecal collection experiments were carried out, one in July 1989 (with three common and three pygmy shrews) and the other in March 1990 (using two pygmy and four common shrews). The shrews used in the experiments had been trapped in the field 1-3 days previously and were autopsied at the end of the experiment so that the faecal output could be related to the worm burden.

Faecal samples were weighed as soon as possible (using a Sartorius balance accurate to four decimal places) to minimise the effects of drying on their mass and stored in a plastic specimen tube in a freezer.

Examination of Faecal Samples

Several methods were investigated in an attempt to develop a satisfactory

technique: A modified McMaster technique, an alternative flotation technique, an examination of the whole sample using successive 0.1ml aliquots, and a dilution count method.

Flotation Techniques

Modified McMaster Method

(1) Faecal samples were weighed when fresh and then frozen for later examination.

(2) The sample, together with a small amount of physiological saline, was ground up in a glass pestle and mortar, sieved through 0.5mm mesh and the pestle and mortar rinsed with saline. This process removed larger impurities whilst the smaller impurities and helminth eggs were washed into a graduated centrifuge tube. The faecal sample often contained a small residue of bedding from the traps, if present this was dried and its mass subtracted from the original mass of the faeces.

(3) Each sample was centrifuged at 2000 rpm for five minutes and the supernatant poured off.

(4) Sufficient saturated salt solution (either sodium chloride or zinc sulphate solution) was added to the centrifuge tube to make up the total volume to 2ml. The tube was shaken vigorously and the resulting suspension transferred to three McMaster chambers using a Pasteur pipette and left to stand for 15 minutes.

(5) The number of eggs of each type present under the grid (marked on top of the McMaster chamber) was counted and the number of eggs per gramme of sample was calculated using the following equation:

$$\text{Eggs per gramme (E.P.G.)} = \frac{xy}{0.15m}$$

Where x = the mean number of eggs per grid

y = the total volume of the faeces/salt solution mixture (2ml)

0.15 = volume contained directly below the grid

m = the mass of faeces taken

Alternative Flotation Method

Steps (1), (2) and (3) were followed as above.

(4) The centrifuge tube was filled with saturated zinc sulphate solution so that a positive meniscus was formed on top of the tube. A 22x22mm coverslip was then placed on top of the tube. The tube was left to stand for 15 minutes.

(5) After fifteen minutes the coverslip, along with a drop of salt solution was carefully removed and lowered onto a microscope slide. The eggs which had adhered to the coverslip were then counted under a microscope. The number of eggs per gramme of sample was calculated as follows:

$$\text{E.P.G.} = \frac{\text{number of eggs present}}{\text{mass of sample in grammes}}$$

Non-Flotation Techniques

Examination of the Whole Sample

Steps (1), (2) and (3) were again followed.

(4) Successive 0.1ml aliquots of physiological saline were added to the remaining

pellet using a Socorex Micropipette 811/821. After thorough mixing, 0.1ml samples were drawn off.

(5) The samples were pipetted onto a slide and the eggs present were identified and counted under the microscope.

(6) Steps (4) and (5) were repeated until virtually all the eggs present in the sample had been removed - this could be seen by a sharp decrease in the number of eggs being counted.
About five aliquots were required.

(7) For each type of egg the total count was divided by the faecal weight (grammes) to calculate the number of eggs per gramme of faeces.

Dilution Count Method

Steps (1), (2) and (3) were followed as described above.

(4) Physiological saline was added to the pellet in the centrifuge tube until the total volume was equal to 2ml.

(5) The tube was then shaken vigorously and a 0.1ml sample of the suspension withdrawn using the Socorex micropipette.

(6) The 0.1ml sample was pipetted onto a microscope slide, covered with a 22x22mm coverslip and the number of each type of egg was counted under a microscope.

(7) Steps (5) and (6) were repeated twice, giving three replicate slides.

(8) The number of eggs present per gramme of faeces was calculated as follows:

$$E.P.G. = \frac{20x}{m}$$

Where x = the mean number of eggs counted per slide

m = the mass in grammes of the faecal sample

20 = the number of 0.1ml aliquots which are contained in a 2ml suspension

In the faecal collection experiments the number of eggs produced by each parasite species in twenty four hours (eggs per day or E.P.D.) was calculated using the following formula:

$$E.P.D. = 120x$$

Where x = the mean number of eggs per slide

120 = 20 (the number of 0.1ml aliquots in the 2ml suspension) **x** 6 (the number of hours in a day [24] ÷ the number of hours over which the sample was collected [4]).

Results

(A) Identification of Eggs

From the prevalence data given in Chapter 6 it could be inferred that eggs from the following helminths were most likely to appear in faecal samples: Cestodes - *Choanotaenia crassiscolex, Hymenolepis schaldybini, H.scutigera* and *H.furcata;* Nematodes - *Longistriata spp., Parastrongyloides winchesi, Stefanskostrongylus soricis* and *Eucoleus oesophagicola;* Digeneans - *Brachylaemus fulvus* and *Dicrocoelium soricis.*

The dimensions of these eggs are given in Table 4.1 below:

Table 4.1. Dimensions of helminth eggs present in *Sorex araneus* and *S.minutus* in Britain.

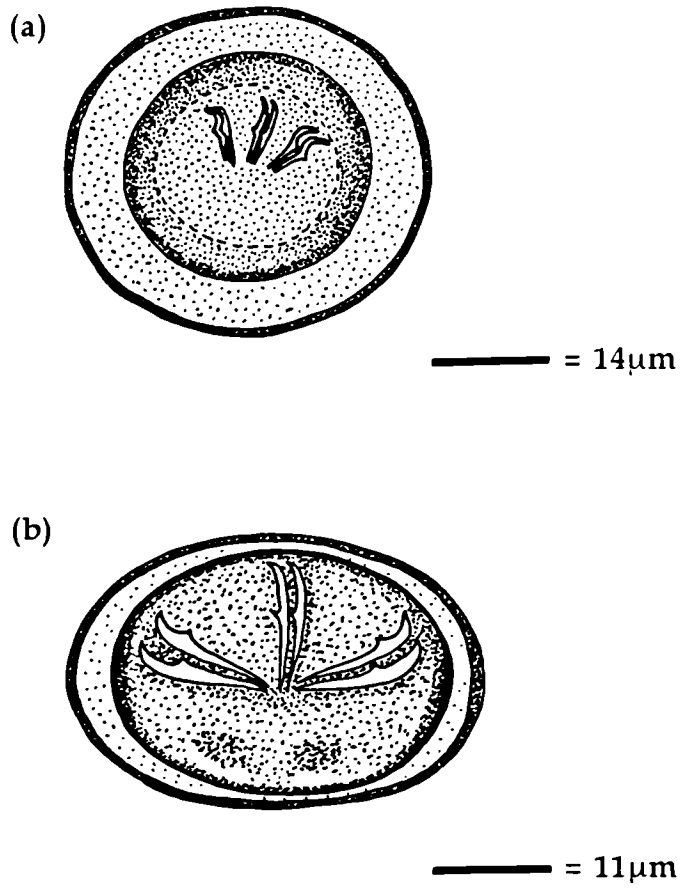
Species	Egg Length (μm)	Egg Width (μm)
Choanotaenia crassiscolex	46-54	27-31
Hymenolepis schaldybini	77-97	47-86
Hymenolepis scutigera	33-57	27-52
Hymenolepis furcata	37-45	29-39
Longistriata spp.	44-60	24-44
Parastrongyloides winchesi	44-65	27-42
Eucoleus oesophagicola	64-82	27-34
Stefanskostrongylus soricis	54-74	32-49
Brachylaemus fulvus	27-34	15-17
Dicrocoelium soricis	35-40	20-27

Cestodes

In addition to size differences, the eggs of cestodes most commonly found in *S.araneus* and *S.minutus* can be distinguished by easily visible features. Eggs of *C.crassiscolex* possess a heavily pigmented membrane enclosing the embryophore. The size of the embryophore of *H.schaldybini* is large in proportion to the dimensions of the outer membrane of the egg (Plate 4.1). The eggs of *H.scutigera* are almost spherical with the spherical embryophore in the centre (Figure 4.1), while those of *H.furcata* are elliptical with the width of the embryophore almost as large as that of the egg itself (Figure 4.1, Plate 4.1).

Plate 4.1 shows that the eggs of *H.schaldybini* and *H.furcata* obtained from faecal samples could readily be identified with those taken from gravid proglottids.

Figure 4.1. Eggs of (a) *Hymenolepis scutigera*, (b) *H.furcata*.



Nematodes

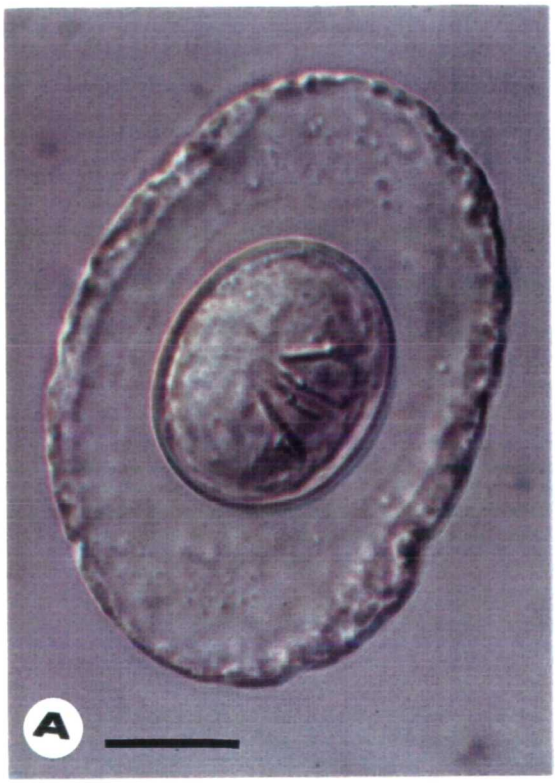
The eggs of *Longistriata spp.* and *Parastrongyloides winchesi* could not be distinguished since they were similar in size and appearance. Any slight differences in appearance of the two types of egg were obscured by the fact that the appearance of the eggs altered as the larvae inside them developed. Since the eggs of *Longistriata spp.* and *Parastrongyloides winchesi* could not be distinguished from each other they were grouped together as "nematode" eggs.

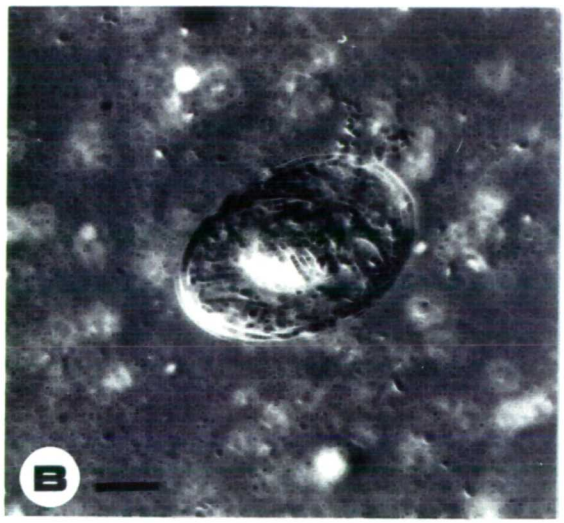
Eucoleus oesophagicola belongs to the sub-family Capillariinae whose eggs are recognized by the presence of a polar plug at either end (Plate 4.2). Eggs of this species are larger than those of the less common *Liniscus incrassatus* (64-82 x 27-34μm compared to 50-60 x 22-27μm).

The L1 larvae of *Stefanskostrongylus soricis* developed inside the eggs before they were released by the female worm. Hence the L1 larvae rather than the eggs of this species would be found in the faecal samples. The larvae (Plate 4.1) measure 230-350μm in length with a diameter of about 10μm.

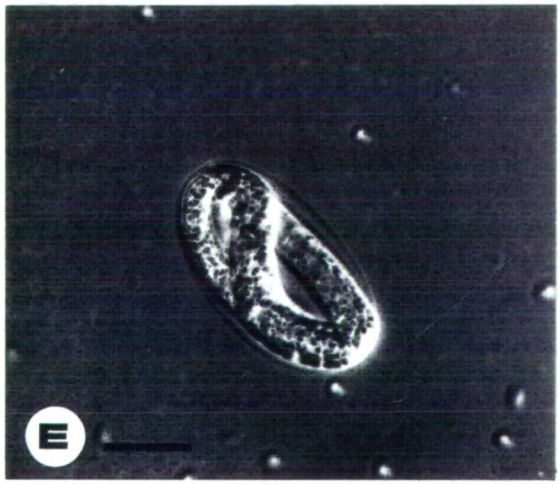
Digeneans

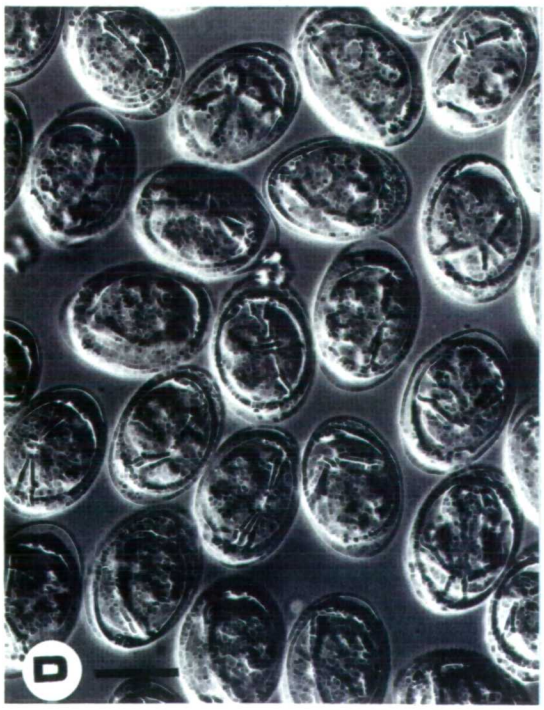
In addition to the size differences between the two species, eggs of *D.soricis* could be distinguished by the presence of two pigmented granules not found in eggs of *B.fulvus* (Plate 4.2).


A

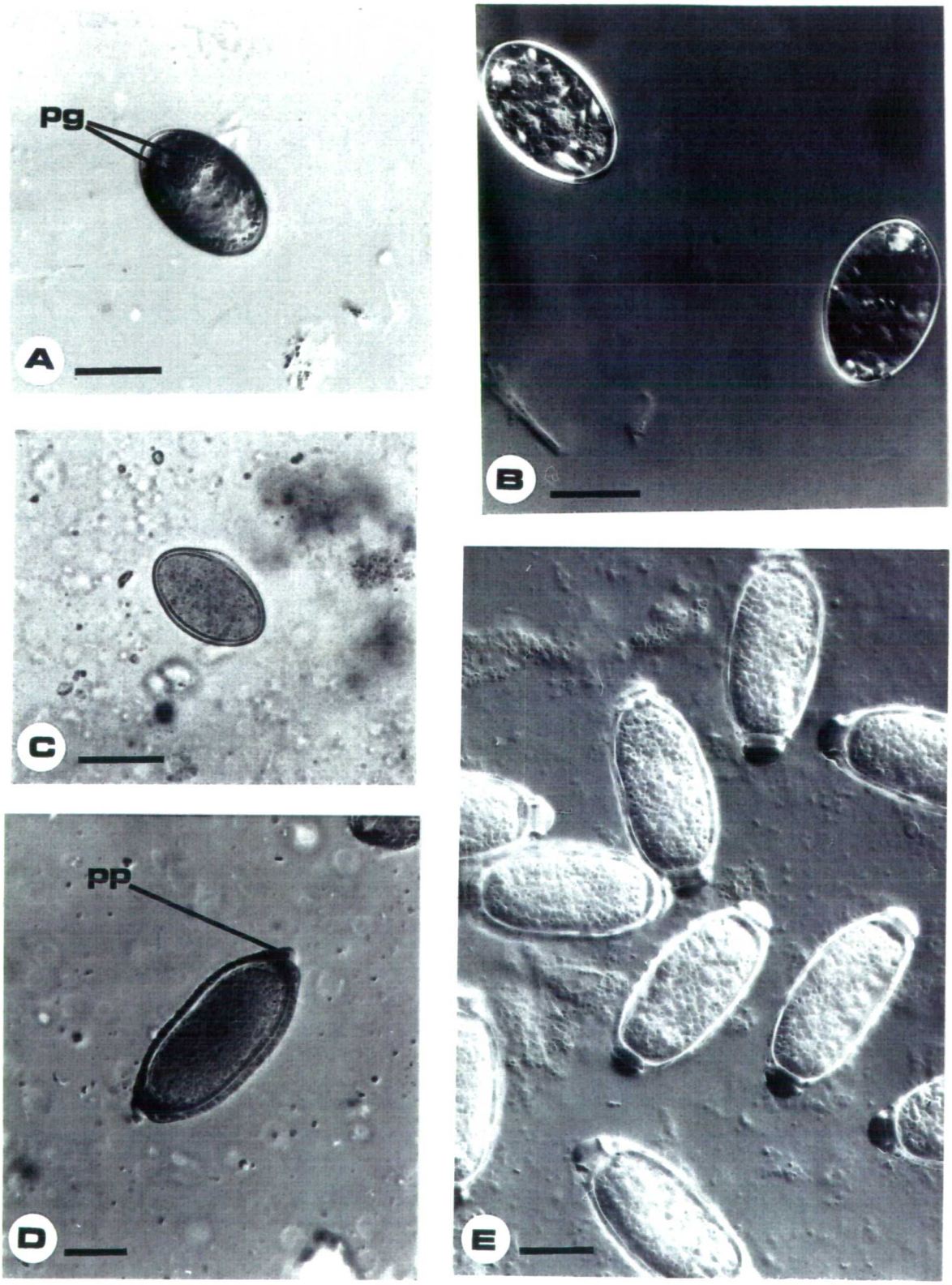

B


C


E


D


F


pg
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(B) Development of a Faecal Analysis Technique

Flotation Techniques - Modified Mcmaster Method

The eggs of most species of helminth (not digeneans) were found to float in saturated sodium chloride solution, but some deformation of eggs occurred.

It was decided to determine whether this deformation affected the number of eggs recovered. Thus for three faecal samples the same suspension of eggs was used to provide samples both for the McMaster method and the dilution count method. All three samples were collected from specimens of *S.araneus* between 6.45-10.45am on 22/7/89. The results are shown in Table 4.2. There was an appreciable difference from the results of the two methods, on average approximately eight times as many eggs being recovered by the dilution count as by the McMaster method.

A further comparison was made between the dilution count method and the McMaster technique using 50% saturated sodium chloride solution (the saturation recommended by Dunn & Keymer (1986) for *Heligmosomoides polygyrus* eggs). The four faecal samples used were collected from specimens of *S.araneus* and *S.minutus* between 2.45-6.45am on 22/7/89. The results of this experiment (Table 4.3) indicated that 50% saturated sodium chloride solution was not a suitable medium for flotation of the eggs.

Table 4.2. Comparison of McMaster method using 100% saturated sodium chloride solution, and dilution count method.

Sample	Egg type	McMaster count (E.P.G.)	Dilution count (E.P.G.)
Sa K2[1]	*L.incrassatus*	14	63
	C.crassiscolex	14	125
	Nematode	472	5250
Sa K1	Nematode	3064	19864
Sa K3	Nematode	412	2195
	L.incrassatus	46	686
	E.oesophagicola	0	412

[1] Codes were assigned to the shrews during the course of the present study. The first two letters refer to the species of shrew ('Sa' = *S.araneus*, 'Sm' = *S.minutus*), the letter(s) after this refer to the sample of shrews (eg. 'K' = Lipper Pond, July 1989); the number '2' indicates that the shrew was the second one in the sample to be dissected.

Table 4.3. Comparison of McMaster method using 50% saturated sodium chloride solution, and dilution count method.

Sample	Egg type	McMaster count (E.P.G.)	Dilution count (E.P.G.)
Sm L2	Nematode	196	63
	H.furcata	0	20577
	H.schaldybini	0	1664
Sm L3	Nematode	2368	21163
	H.furcata	0	31522
	H.schaldybini	0	1036
	D.soricis	230	1628
Sa K2	Nematode	0	18803
	L.incrassatus	0	372
	C.crassiscolex	0	1489
	H.schaldybini	0	186
Sa K1	Nematode	91	39204

Alternative Flotation Method

A mature specimen of *Brachylaemus fulvus* (which had been preserved in 70% alcohol) containing about 190 eggs was ground up and treated according to the alternative flotation method.

The coverslip was removed after 10 minutes and replaced by another, after 10 further minutes this was also removed.

No eggs were found.

In a further three experiments using a known number of *B.fulvus* eggs the alternative flotation method was followed, the coverslip being removed at intervals and being replaced with another one.

The cumulative percentages of eggs recovered with time are shown in Table 4.4.

Table 4.4. Percentage of eggs recovered using the alternative flotation technique.

Time/mins	Percentage of Eggs Recovered		
	Expt (1)	Expt (2)	Expt (3)
10	3.0	1.6	-
20	4.8	-	2.2
30	-	6.0	-
40	-	-	4.2
57	21.8	-	-
60	-	6.8	5.5
85	-	7.9	6.4
95	30.0	-	-
160	-	-	7.9

Non-Flotation Techniques

Examination of the Whole Sample

Using this technique, faecal samples from seven shrews were examined. Eggs of *Hymenolepis schaldybini, H.scutigera, Eucoleus oesophagicola, Liniscus incrassatus,* Nematoda (i.e. *Parastrongyloides winchesi* or *Longistriata spp.*) and *Brachylaemus fulvus* were identified.

Use of this technique was discontinued since it was exceedingly time-

consuming as thousands of eggs were sometimes found in a sample.

Dilution Count Method

Accuracy of the Technique

In order to assess the accuracy of the technique for estimating the number of eggs present in the sample, three 0.1ml samples were counted from each sample and the counts compared. Tables 4.5 to 4.9 show the results of this experiment. All faecal samples were collected between 10.45am and 2.45pm on 21/7/89 from *S.araneus* and *S.minutus* kept in the laboratory.

Table 4.5. Faecal sample from Sm L2 - Numbers of helminth eggs counted in three successive 0.1ml subsamples using the dilution count method.

Slide	Parasite Egg		
	H.furcata	Nematode	*H.scutigera*
1	28	171	5
2	22	150	8
3	27	161	8
MEAN:	25.67	160.67	7.00
S.D.[1]:	3.21	10.50	1.73
C.V.[2]:	12.52	6.54	24.74

[1] S.D. = Standard deviation

[2] C.V. = Coefficient of variation.

Table 4.6. Faecal sample from Sm L3 - Numbers of helminth eggs counted in three successive 0.1ml subsamples using the dilution count method.

Slide	Parasite Egg				
	H.furcata	Nematode	*H.scutigera*	*H.schaldybini*	*D.soricis*
1	18	249	4	1	0
2	18	210	1	0	1
3	18	188	2	0	1
MEAN:	18.00	215.67	2.33	0.33	0.67
S.D.:	0.00	30.89	1.52	0.58	0.58
C.V.:	0.00	14.32	65.47	173.21	86.60

Table 4.7. Faecal sample from Sa K2 - Numbers of helminth eggs counted in three successive 0.1ml subsamples using the dilution count method.

Slide	Parasite Egg		
	C.crassiscolex	Nematode	*L.incrassatus*
1	18	107	2
2	13	80	0
3	11	99	1
MEAN:	14.00	95.33	1.00
S.D.:	3.61	13.87	1.00
C.V.:	25.75	14.55	100.00

Table 4.8. Faecal sample from Sa K3 - Numbers of helminth eggs counted in three successive 0.1ml subsamples using the dilution count method.

Slide	Parasite Egg		
	Nematode	*L.incrassatus*	*E.oesophagicola*
1	232	5	7
2	268	8	12
3	301	8	9
MEAN:	267.00	7.00	9.33
S.D.:	34.51	1.73	2.52
C.V.:	12.93	24.74	26.96

Table 4.9. Faecal sample from Sm L1 - Numbers of helminth eggs counted in three successive 0.1ml subsamples using the dilution count method.

Slide	Parasite Egg		
	Nematode	*S.soricis*	*D.soricis*
1	88	23	0
2	72	19	3
3	78	23	0
MEAN:	79.33	21.67	1.00
S.D.:	8.08	2.31	1.73
C.V.:	10.19	10.66	173.21

If the results where the mean number of eggs per slide is less than 15.00 are ignored, the mean coefficient of variation is found to equal 10.2%. Hence for eggs present in numbers of greater than 15 per 0.1ml (300 per faecal sample) the technique is sufficiently accurate.

(C) The Relationship Between Faecal Egg Counts and Worm Burden

Faecal Collection Experiments

All faeces produced by the shrews were collected and weighed; the faecal weights are given in Tables 4.10 and 4.11. All the shrews used in these experiments were mature, shrews K2 and K3 were pregnant females.

The amount of faeces produced during each four hour period varied significantly with no obvious periodicity. *S.araneus* produced 1.13 ± 0.41g of faeces per day (24 hours) while *S.minutus* produced
0.75 ± 0.15g per day. The faecal output represented 12.6 ± 3.1% of the body weight of *S.araneus* and 16.3 ± 2.7% of the body weight of *S.minutus*.

Efficiency of the Dilution Count Technique in Detecting the Helminth Species Present in the Host

The number of species of helminths known to produce eggs in the shrew, ie. those species for which the shrew was the definitive host, could be determined when the shrews were dissected. In some cases the parasites would not have been expected to produce eggs as they were either immature or, in the case of dioecious species, only one sex was present. The number of species capable of producing eggs was compared with the number of species identified by the presence of their eggs in the faeces.

It was found that the dilution count technique was about 84% effective in detecting the eggs of species known to be producing them. Overall, the technique detected about 68% of the species whose definitive host was the shrew (Table 4.12).

Table 4.10. Faecal output by six individual shrews over a 28 hour period.

Time	Mass of Faeces Produced (g)					
	Sm L2	Sm L3	Sm L1	Sa K1	Sa K2	Sa K3
21/7/89						
10.45am - 2.45pm	0.1040	0.0839	0.1005	0.2006	0.2151	0.2158
2.45pm - 6.45pm	0.0993	0.1481	0.1137	0.2033	0.4073	0.2839
6.45pm - 10.45pm	0.1439	0.1567	0.1055	0.1430	0.2545	0.2311
10.45pm - 2.45am (22/7)	0.1993	0.2009	0.0777	0.1942	0.5230	0.3102
2.45am - 6.45am	0.1635	0.1419	0.1030	0.1538	0.2318	0.1128
6.45am - 10.45am	0.2085	0.1403	0.0896	0.1762	0.2916	0.3200
10.45am - 2.45pm	0.1233	0.1145	0.0588	0.2239	0.1903	0.2553
Total	1.4018	0.9863	0.6488	1.2950	2.1136	1.7291
Mean	0.1488	0.1409	0.0927	0.1850	0.3019	0.2470
S.D.	0.0437	0.0362	0.0190	0.0288	0.1206	0.0707
Faecal output (g/day)	0.0893	0.8454	0.5561	1.1100	1.8117	1.4821
Faeces as % of body weight	18.9	18.8	13.5	12.8	16.2	12.0

Table 4.11 Faecal output by six individual shrews over a 28 hour period.

Time	Mass of Faeces Produced (g)					
	Sm FF4	Sm FF3	Sa AA3	Sa AA1	Sa BB2	Sa BB1
28/3/90						
6.45am - 10.45am	0.1901	0.1465	0.1029	0.0981	0.0966	0.0789
10.45am - 2.45pm	0.0892	0.2223	0.2146	0.1934	0.2183	0.0695
2.45pm - 6.45pm	0.1211	0.2003	0.2387	0.2049	0.1466	0.0895
6.45pm - 10.45pm	0.1144	0.0478	0.2144	0.1435	0.1137	0.0964
10.45pm - 2.45am (29/3)	0.0855	0.0979	0.2246	0.1175	0.1161	0.1246
2.45am - 6.45am	0.0362	0.1382	0.2363	0.0977	0.1111	0.0877
6.45am - 10.45am	0.0491	0.1799	0.3352	0.1364	0.1136	0.1068
Total	0.6856	1.0329	1.5667	0.9915	0.9160	0.6534
Mean	0.0979	0.1476	0.2238	0.1416	0.1309	0.0933
S.D.	0.0512	0.0604	0.0678	0.0431	0.0414	0.0182
Faecal output (g/day)	0.5877	0.8853	1.3429	0.8500	0.7851	0.5601
Faeces as % of body weight	12.6	17.5	17.0	12.4	10.0	7.5

Table 4.12. Effectiveness of the faecal analysis technique in detecting the presence of helminth eggs

Shrew	(a) Maximum No. of species which could be detected if all were mature.	(b) No. of species known from autopsy to be mature.	No. of species detected	% of (a) detected	% of (b) detected
Sm L1	4	3	3	75	100
Sm L3	4	4	4	100	100
Sm L1	4	3	3	75	100
Sa K1	5	2	2	40	100
Sa K3	6	3	3	50	100
Sa K2	6	5	4	67	80
Sm FF4	4	4	3	75	75
Sm FF3	3	3	3	100	100
Sa AA3	5	3	1	20	33
Sa AA1	5	2	1	20	50
Sa BB2	4	2	2	50	100
Sa BB1	6	4	3	50	75
Total	56	38	32	68%	84%

Helminth Fecundity

The number of eggs per day (E.P.D.) produced by each helminth species in each shrew is shown in Tables 4.13 and 4.14. The mean number of eggs produced by each mature digenean or cestode and each mature female nematode is also shown. It can be seen that there was no relationship between number of mature worms and number of eggs produced per day.

In order to examine whether there was a relationship between fecundity and the density of the parasite in the host, the number of eggs per nematode per day was plotted against the number of nematodes present in the host; no relationship was observed (Figure 4.2).

Rhythms of Egg Output

In order to investigate any circadian rhythm in egg output, graphs of egg output against time were plotted for some of the helminths. The egg counts for each faecal sample were expressed as E.P.D. For the nematode egg counts in the March 1990 experiment, values of E.P.G. have also been plotted. These results are shown in Figures 4.3 to 4.7.

The graphs of egg output against time indicate no circadian rhythms of egg output in nematodes or cestodes; egg output varies unpredictably from sample to sample. The graphs for E.P.G. against time are similar to those for E.P.D. against time.

Table 4.13. Estimation of the fecundity of nematodes and digeneans using faeces collected over 28 hours.

Helminth	Shrew	E.P.D. (mean value)	No. mature worms	Eggs/worm /day
"Nematode"	Sm L2	14140	11	1285
"Nematode" *	Sm L3	20458	22	930
"Nematode"	Sm L1	12990	22	590
"Nematode"	Sa K1	32982	103	320
"Nematode"	Sa K3	12154	200	61
"Nematode"	Sa K2	12609	61	207
"Nematode"	Sm FF4	314	4	79
"Nematode" *	Sm FF3	686	6	114
"Nematode" *	Sa AA3	1062	20	53
"Nematode" **	Sa AA1	680	2	340
"Nematode" *	Sa BB2	2474	27	92
"Nematode"	Sa BB1	1210	20	61
L.incrassatus	Sa K2	143	7	20
L.incrassatus	Sa K3	802	11	73
E.oesophagicola	Sa K3	664	3	221
E.oesophagicola	Sm FF4	291	0	-
E.oesophagicola	Sa BB2	29	1	29
S.soricis[1]	Sm L1	3662	4	916
D.soricis	Sm L3	811	3	270
D.soricis	Sm L1	56	3	19
D.soricis	Sm FF3	12	2	6

* = only *Longistriata spp.* present

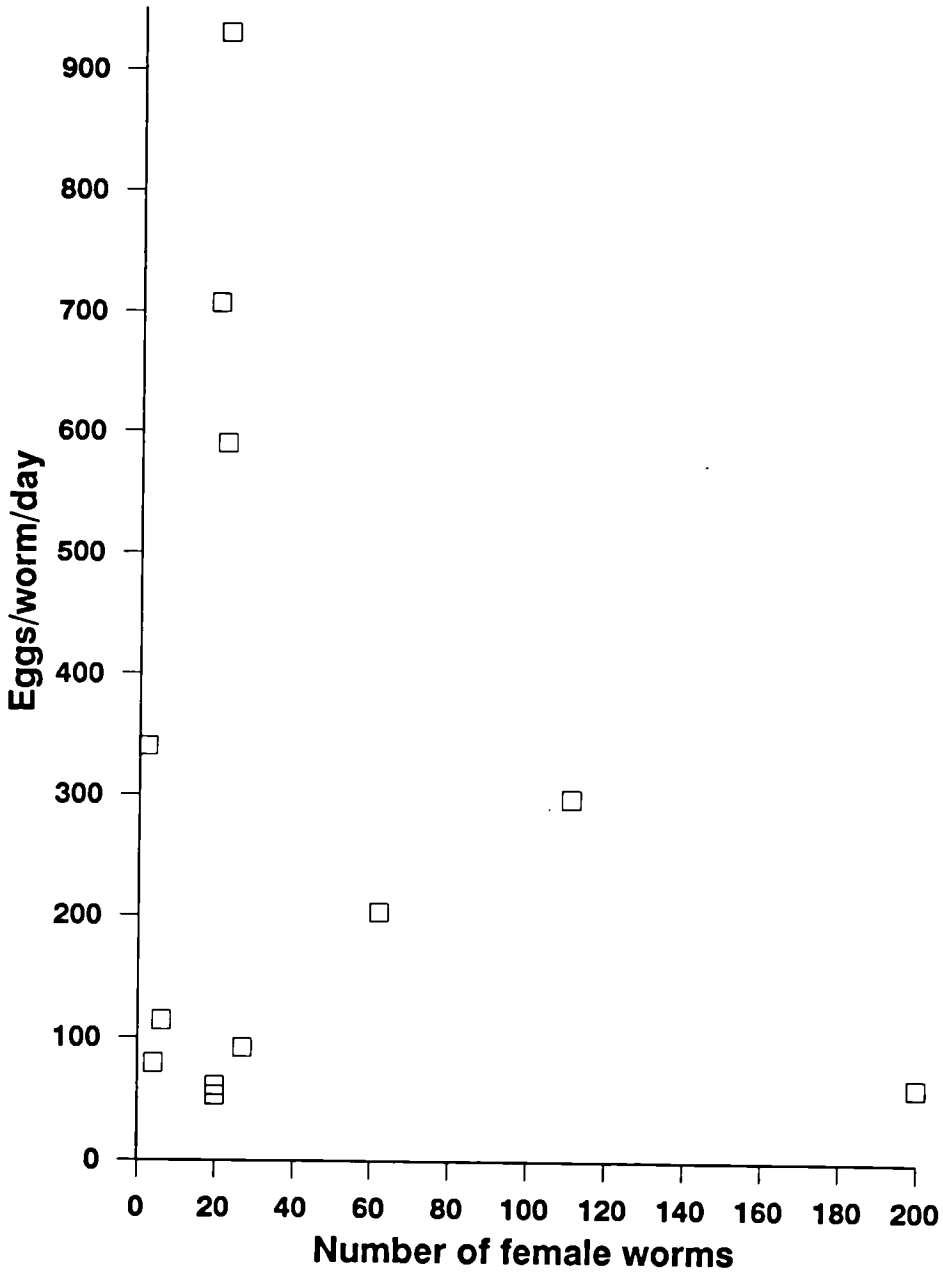
** = only *Parastrongyloides winchesi* present

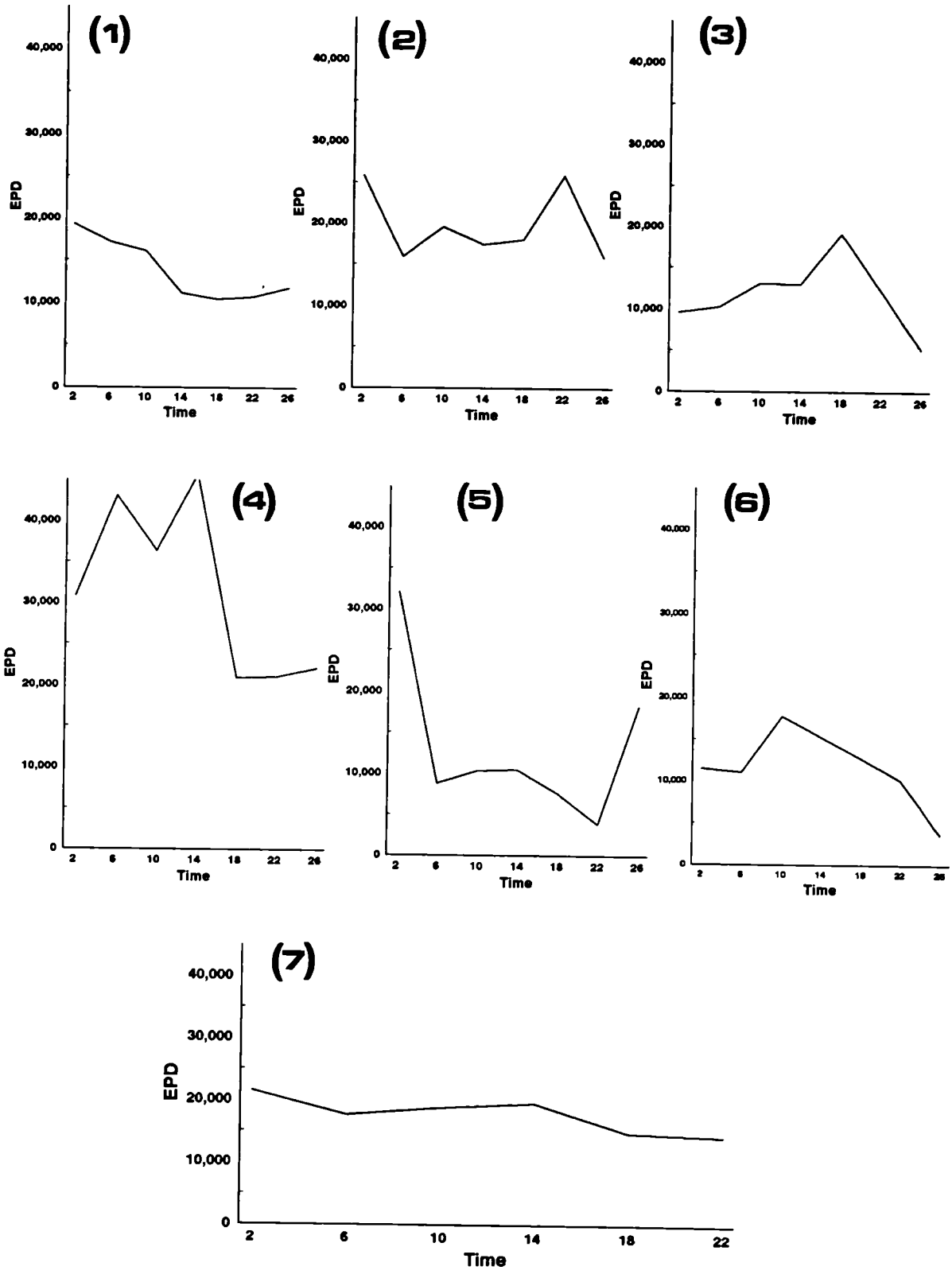
others had both species present

[1] *Stefanskostrongylus soricis*

Table 4.14. Estimation of fecundity of cestodes using faeces collected over 28 hours.

Helminth	Shrew	E.P.D. (mean value)	No. mature worms	Eggs/worm /day
C.crassiscolex	Sa K2	2452	1	2452
C.crassiscolex	Sa BB1	1533	3	511
H.furcata	Sm L2	13192	3	4397
H.furcata	Sm L3	11772	1	11772
H.furcata	Sm FF3	16046	1	16046
H.schaldybini	Sm L2	1929	23	84
H.schaldybini	Sm L3	4896	6	816
H.schaldybini	Sa K1	162	1	162
H.schaldybini	Sa K2	31	8	4
H.schaldybini	Sm FF4	137	3	46
H.schaldybini	Sa BB1	252	4	63


Eggs/worm/day
900
800
700
600
500
400
300
200
100
0
0
20
40
60
80
100
120
140
160
180
200
Number of female worms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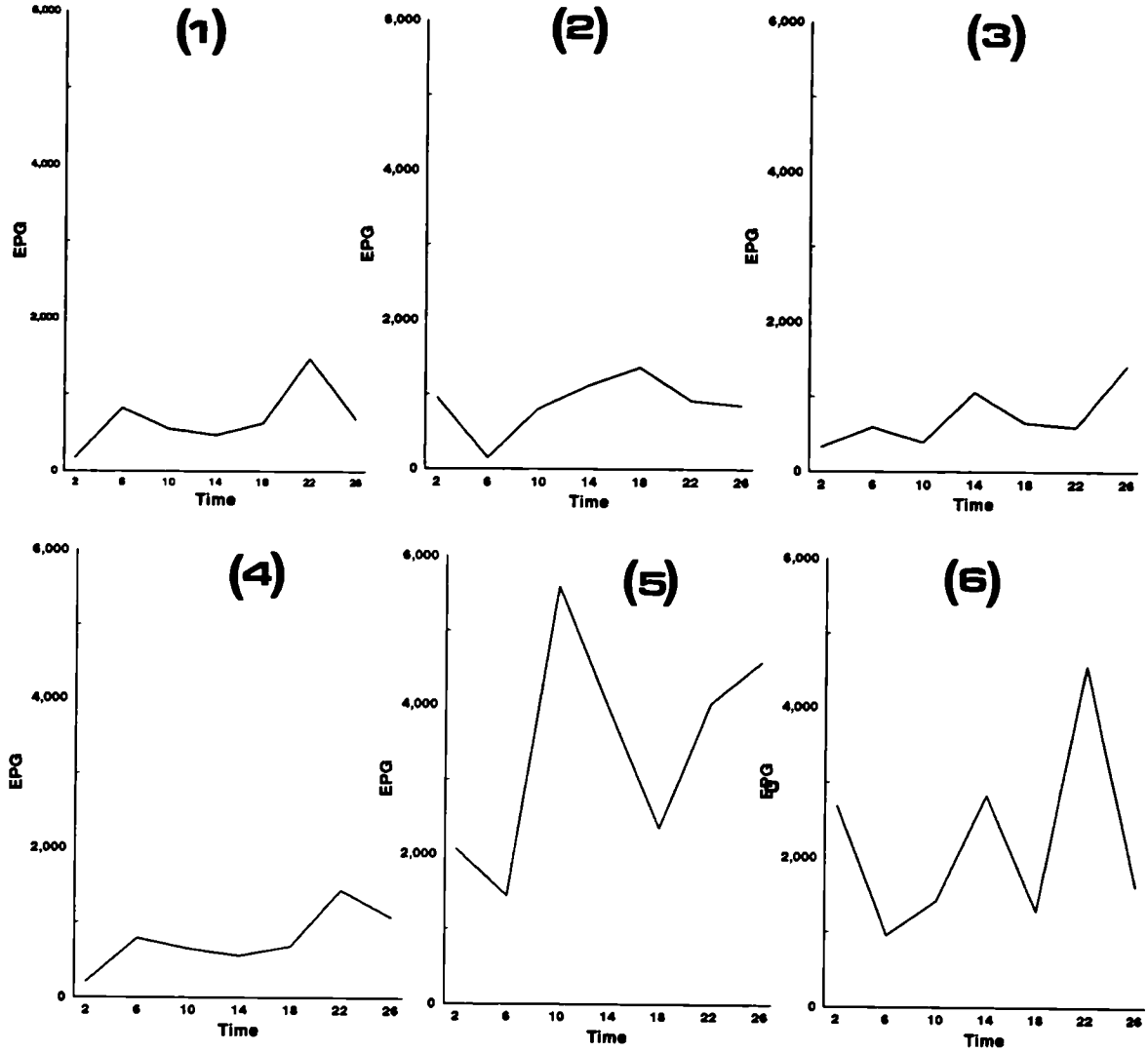
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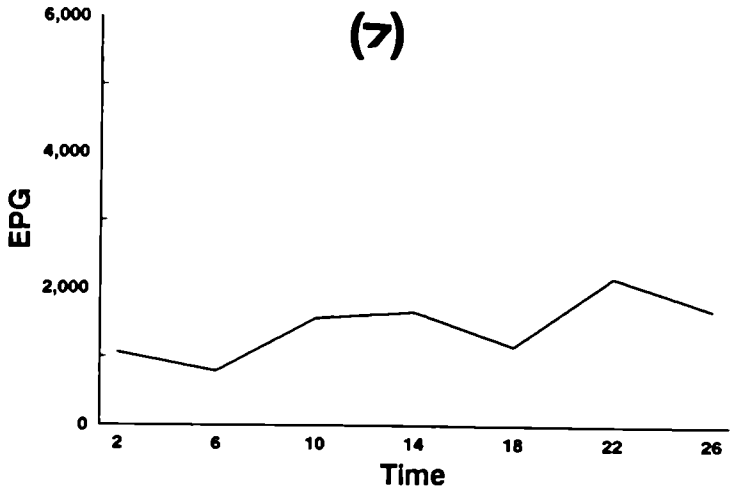
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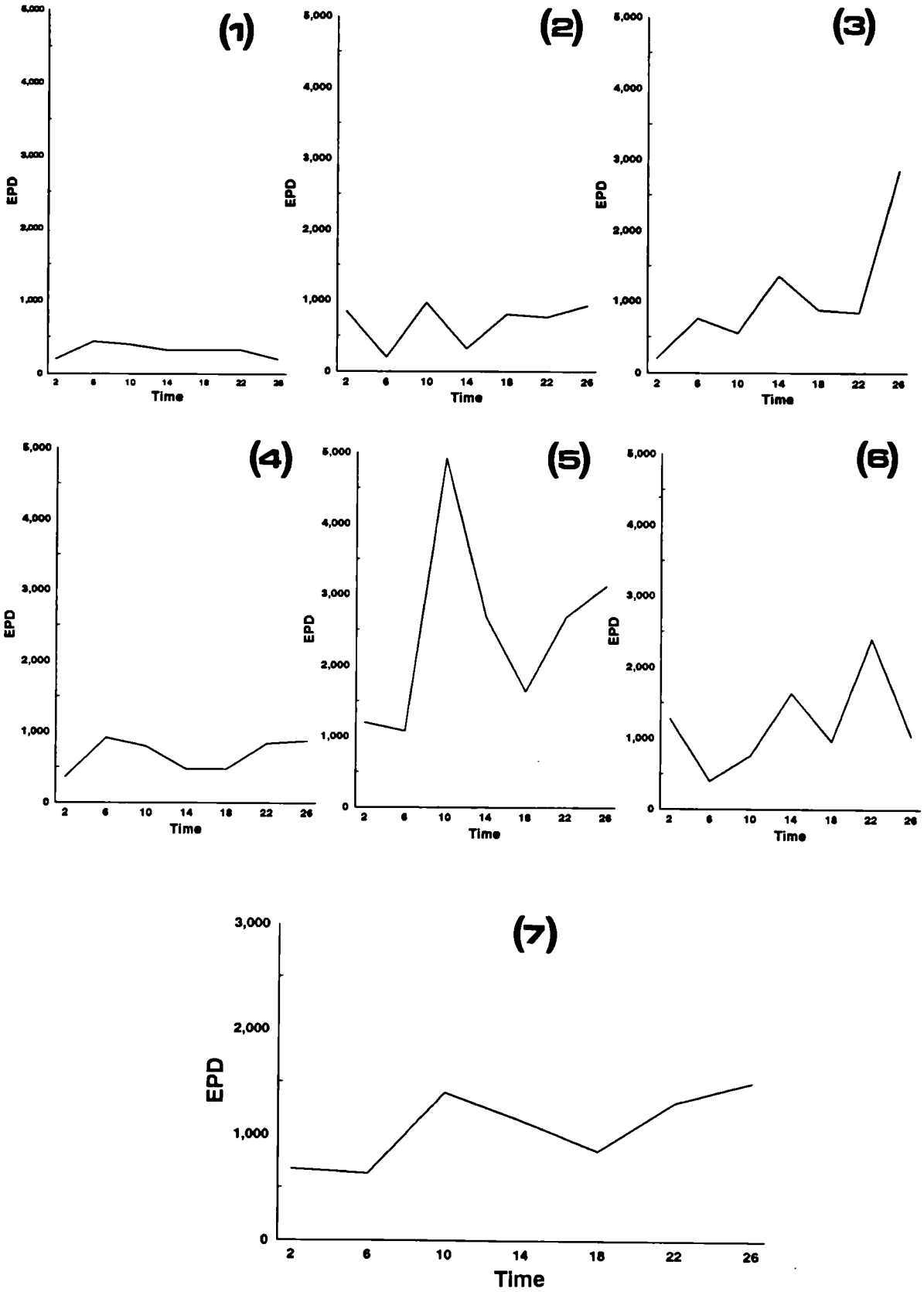
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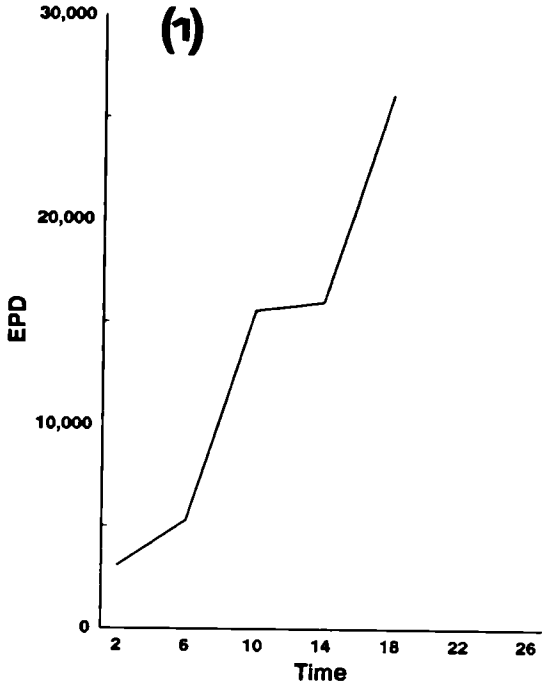
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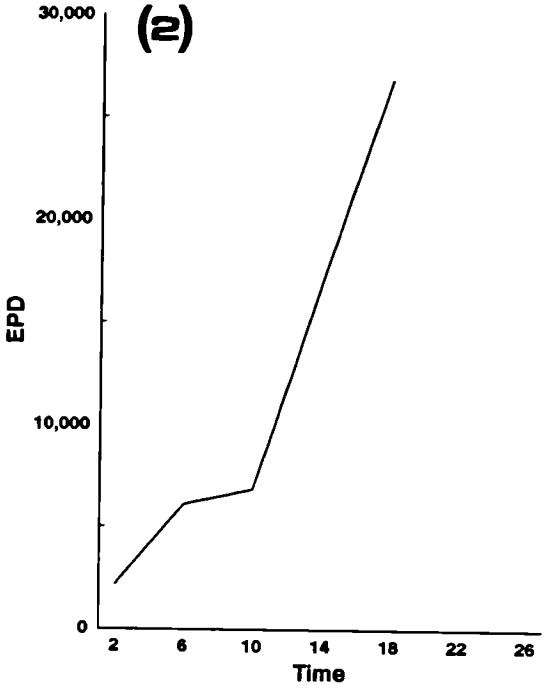
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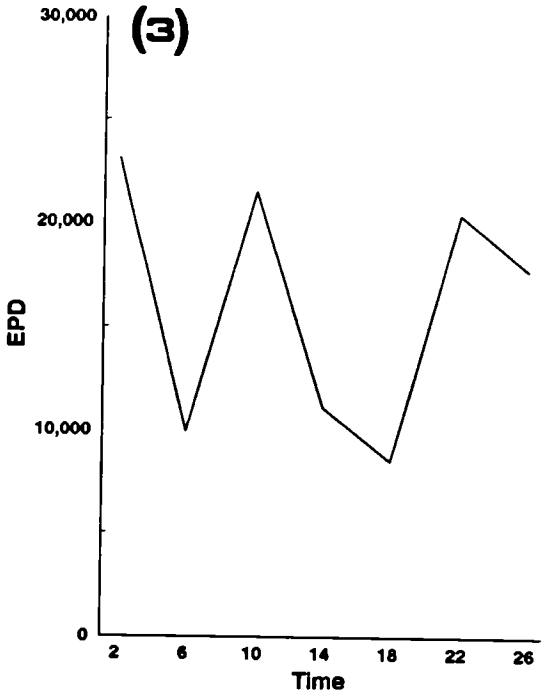
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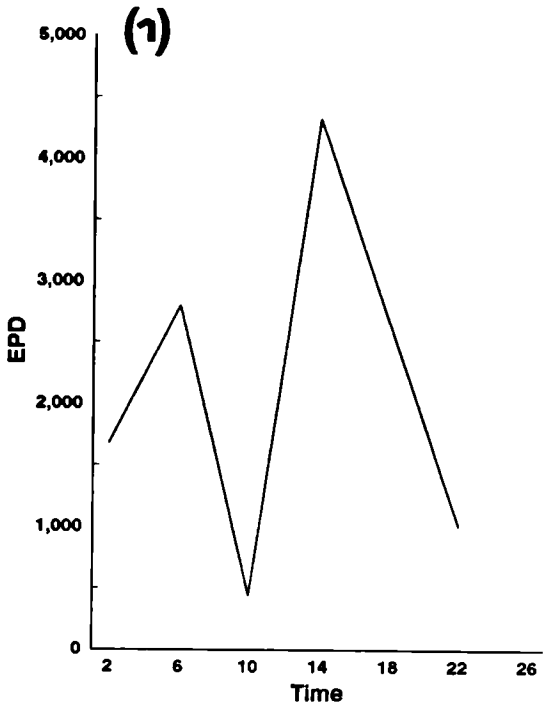
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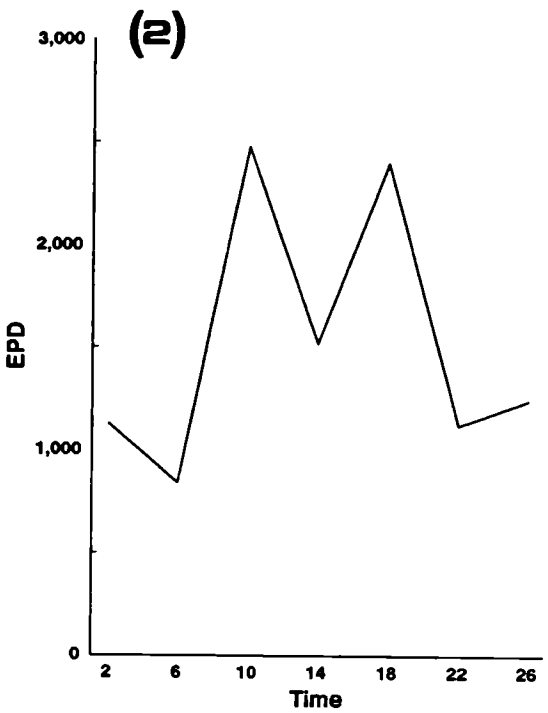
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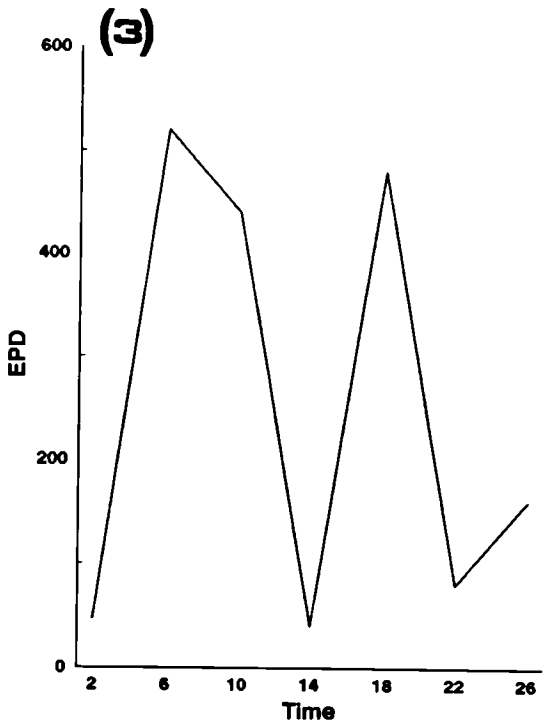
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Discussion

Identification of Eggs

The dimensions of helminth eggs from shrews as given in the literature show considerable variation because of differences in the methods adopted in egg measurement (Chapter 3). Hence comparison of eggs recovered by faecal analysis techniques with descriptions in the literature may not be a reliable means of identification. The passage of eggs through the intestine and the processes used in the faecal analysis technique may cause structural changes in the eggs or they may hatch, e.g. in *Stefanskostrongylus soricis* in the present study, so that the larvae rather than the eggs are recovered from the faecal samples.

It is important, therefore, to compare the eggs obtained from faecal samples with those obtained from mature worms. In the present study it was demonstrated that the eggs of most helminth species were sufficiently similar in the faecal samples to those in the mature worms to allow helminth eggs found in the faeces to be positively identified.

The only major difficulty in egg identification was in distinguishing between eggs of *Longistriata spp.* and *Parastrongyloides winchesi.* The difficulty in differentiating between the eggs of different nematode species was also noted by M.A.F.F. (1986). It may perhaps be possible to distinguish between similar egg types using a staining technique, but it was not feasible to investigate this in the present study.

Development of a Faecal Analysis Technique

A direct comparison of the McMaster and dilution count techniques showed that approximately eight times as many eggs were recovered using the latter method. This large discrepancy could be due either to a huge over-estimate of egg numbers when using the dilution count technique or an underestimate of numbers when using the McMaster technique.

Inaccuracies in estimation of the number of eggs per gramme in a faecal sample could be caused by the following 6 factors:

(1) Inaccuracies in weighing. In the present study the faecal samples could be weighed to four decimal places, but because of the problem of water loss from the samples and loss of faeces in the weighing process, the fourth and possibly the third decimal place were unlikely to have been reliable.

(2) Inaccuracies in volume measurement. The volume of the faecal suspension made by adding the salt solution to the faecal pellet in the centrifuge tube was measured by graduations on the side of the tube, the accuracy of which was checked by adding known volumes of solution from a pipette. Since the graduations were in divisions of 0.1ml, the largest error expected in the volume measurement would be about 0.05ml (half a division). i.e. an error of 2.5%.

Any errors in the weighing and volume measurement would apply equally to the McMaster and dilution count techniques so when these two methods were being compared the above sources of error could not have been responsible for any observed differences in egg counts.

(3) Uneven distribution of eggs. Helminth eggs may not have been evenly distributed in the faeces, but since all the faeces were used in each sample this would not have been a source of error as long as the suspension was adequately homogenized. When the McMaster and dilution count techniques were compared, the consistently higher values obtained by the latter technique could not have been due to uneven distribution of eggs in the faecal suspension. The calculation of standard deviations and coefficients of variation of several sets of three 0.1ml aliquots examined using the dilution count method (Tables 4.5 to 4.9), indicates that the eggs were evenly distributed in the suspension.

(4) Inaccurate measurement of the subsample taken for counting would have been very unlikely to occur when the McMaster technique was employed since the volume was measured using a grid already marked out on the McMaster slide. Any eggs within the boundaries of the grid are known to come from a volume of exactly 0.15ml. In the dilution count technique the volume examined was measured using a Socorex micropipette whose accuracy was stated by the manufacturer to be between 0.08 and 0.34% so as long as the pipette was used correctly and no debris was stuck in the pipette tip, the volume delivered would be accurately measured.

(5) Inaccurate counting of the actual number of eggs present under the coverslip or McMaster grid is unlikely as in both cases the areas were examined systematically by a fixed route on each occasion. Although the coverslip did not have a grid marked on it to aid counting, the number of eggs could be counted accurately since Stoll & Hausheer (1926) found that two experienced operators both count the same number of eggs using this method.

(6) Loss of, or damage to eggs. Although the specimen tube, the sieve and glass pestle and mortar were all washed thoroughly, there is a possibility that a small number of the eggs might have been lost in steps (1) and (2) of the faecal analysis technique. There is also the possibility of eggs adhering to the side of the centrifuge tube during homogenisation of the faecal pellet and salt solution. Eggs might also adhere to the pipette during transfer to the slide or McMaster chamber.

Such losses of eggs would be similar in both techniques. A possible explanation for the much smaller number of eggs delivered using the McMaster technique when compared with the dilution count method is that the eggs for some reason failed to float in the salt solution used. This may have been due to egg damage caused by osmosis, the salt solution causing water to flow out of the eggs, leading to the outer membrane becoming crinkled. Deformation of eggs was observed in the present study, especially when a zinc sulphate solution was used for flotation. Zinc sulphate, with its high specific gravity, was necessary to allow eggs of digeneans to float, but caused such a severe deformation of other eggs that they could not be reliably identified. Deformation of eggs caused by saturated sodium chloride solution was observed by Stoll & Hausheer (1926) and Dunn & Keymer (1986) suggested that even 50% saturated sodium chloride might cause damage to nematode eggs.

Deformation of eggs might make them less able to float in the salt solution and the effect is likely to be most significant on those eggs (such as cestodes) whose density is only just low enough to allow them to float in the salt solution. It could be argued that the freezing of the faecal samples damaged the eggs, but eggs from frozen samples placed in physiological saline and examined by the dilution count technique did not appear to be damaged.

Failure of eggs to float in the salt solution, possibly due to damage, seems to

be the main factor responsible for the low egg counts from the McMaster slides.

The percentage of eggs recovered using the McMaster technique seems to have been largely ignored in the literature, but Richards (1991) used a modified direct centrifugal flotation technique to investigate the efficiency of three salt solutions. He added a known number of eggs of the nematode *Toxocara canis* to faecal samples from uninfected foxes and determined the percentage of eggs recovered. When saturated sodium chloride was used, only 13-20% of the eggs were recovered. The maximum recovery rate was 48% using a saturated solution of magnesium sulphate. In experiments on mice infected with *Heligmosomoides polygyrus*, Abu-Madi (unpublished) found that egg counts made using the dilution count technique were consistently higher than those made with the McMaster technique although the discrepancy between the two counts was much less than in the present study.

The dilution count technique was found to be clearly the best of the methods used. A direct smear or similar technique was not practical due to the high concentrations of eggs in the sample. The salt solution used in flotation techniques caused considerable deformation of eggs making identification difficult and decreasing the number of eggs recovered. The only significant inaccuracy in the dilution count technique is likely to be the loss of water from faecal samples if allowed to dry out too much before weighing.

Relationship Between Faecal Egg Counts and Worm Burden

The variability in faecal output between successive samples from the same shrew and between different individual shrews suggests that it would be difficult to relate E.P.G. to E.P.D. Phillipson (1974) suggested that if egg production by female *Aspicularis tetraptera* (Nematoda) in mice was constant and the only factor which varied was faecal production, egg concentration would be inversely proportional to the amount of faeces produced. In fact in his experiments E.P.G. was higher when faecal production by the mouse was higher. No obvious relationship was detected between egg production and faecal output in the present study. (For the relationship between egg output of nematodes in SmL2 - figure 4.3 - and faecal output - table 4.10 - correlation coefficient, $r=-0.49$, $P>0.05$.)

In the laboratory experiments it was possible to investigate egg production in terms of E.P.D., thereby eliminating the effect of variations in faecal output. This would obviously not be possible in field studies. Thus a measurement of E.P.G. in one individual at one particular time would not necessarily be equivalent to the same figure obtained from another individual, or from the same individual on another occasion.

The dilution count technique was very reliable in detecting the presence of parasites producing eggs, although the presence of immature parasites which might have been present in large numbers, could not be detected.

However, the technique was completely unreliable as a means of estimating the number of mature digeneans and cestodes or number of mature female nematodes.

Reasonable correlation between E.P.G. and worm burden has been obtained in laboratory studies where hosts of similar age have been infected with single species infections of helminths of the same age (Kerboeuf, 1982). Such a correlation has not been found under less controlled conditions. Watkins & Harvey (1942) found no correlation between the numbers of eggs of various nematode species in the faeces and the number of mature female worms present in silver foxes. Richards (1991) achieved similar results with *Toxocara canis* (Nematoda) in the red fox, *Vulpes vulpes*.

Several factors may affect the fecundity of adult worms and thereby obscure any relationship between number of worms and egg output. In laboratory experiments on mice Kerboeuf (1982) working on *Heligmosomoides polygyrus* (Nematoda) and Jones & Tan (1971) working on *Hymenolepis microstoma* (Cestoda) found that fecundity decreased as density of the parasite increased. This effect could not be demonstrated in the present study, perhaps due to other factors mentioned below.

As well as intraspecific competition, it is likely that interspecific competition also affects fecundity, so that one helminth species affects the fecundity of another. This has apparently not yet been demonstrated in the literature, but Holmes (1961) showed that *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocephala) were smaller and lower in mean weight in concurrent infections

than when the hosts (rats) were infected with a single species of helminth.

Chai et al. (1981) found some correlation between egg output and length of *Ascaris lumbricoides* (Nematoda) in humans although there was still considerable variation in E.P.G. when worms of the same length were present.

Richards (1991) found a positive correlation between number of eggs present in the uteri of female *Toxocara canis* and the length and weight of the worms. However, he did not find any correlation between number of mature female worms and E.P.G.

Keymer & Hiorns (1986b) found that there was a large variability in the fecundity of *Heligmosomoides polygyrus* present in mice harbouring low numbers of worms and attributed this to host heterogeneity caused by genetic differences in immunity.

Host hormones may also affect the fecundity of helminth parasites: Beck (1952) found that egg output of *Hymenolepis diminuta* decreased in male rats which had been castrated and that egg output in these rats was restored when testosterone and progesterone were administered.

From the results of the present study, as well as other research, it appears that E.P.G. can not be used to estimate worm burden, despite the fact that it has frequently been employed for such a purpose. At best, E.P.G. may be used to give an approximate guide to the intensity of infection and as a fairly reliable indication of the helminth species present in a host individual.

Circadian Rhythms in Egg Output

Circadian rhythms of egg output have been demonstrated in some nematode species. Such rhythms are thought to be advantageous to the parasite. For example, most eggs of the mouse pinworm *Syphacia muris* are laid during the day while the mouse is in the nest, thus increasing the likelihood of reinfection of the mouse (Lewis & D'Silva, 1980; Van der Gulden, 1967). Although shrews have cycles of activity over 24 hours (Churchfield, 1990), these do not involve long periods of inactivity as shrews need to feed every two or three hours to prevent

death by starvation; thus these cycles are not likely to be able to be usefully harnessed by helminth parasites to increase the chances of infection. Since it is likely that all helminths of shrews have indirect life cycles, there would be no advantage to the parasite in facilitating the ingestion of eggs by the shrew as the larval stages would need to develop in an intermediate host.

Although helminths of shrews were not expected to exhibit circadian rhythms of egg output it was important to confirm the absence of such rhythms as any cycles present would affect the reliability of egg counts.

Although various factors are known to affect helminth fecundity it is unlikely that any of these would affect rhythms of egg output, hence the present findings can be considered as confirming the lack of periodicity in egg output.

The erratic nature of egg output (Figures 4.3 to 4.7) provides a further explanation for the unreliability of faecal egg counts in estimating the worm burden.

Summary

The eggs of most helminth species present in *Sorex araneus* and *S.minutus* may be identified from faecal samples. The dilution count method developed for use in the present study gives a good indication of the presence or absence of a given helminth species, but gives very little information on the number of individual parasites present.

The egg output of helminth parasites in shrews is erratic and there is no obvious periodicity in the variation in egg output.

It is important to appreciate that faecal analysis techniques are not able to detect immature parasites, which may have considerable pathological effects on the host.

Chapter 5
Life Cycles of Shrew Helminths
Larval Stages in Invertebrate Hosts

Introduction

Sorex araneus and *S.minutus* have been shown to be parasitised by a large number of helminth species: 30 species in Britain and 45 in Europe (Chapter 3). In most of these cases the shrew is the definitive (or final) host, but in two cases (the nematode, *Porrocaecum sp.* and the acanthocephalan, *Gordiorhynchus aluconis*) the shrew acts as a paratenic host, which is a link between an invertebrate intermediate host and a definitive host such as a predatory bird.

This chapter contains an investigation into the identity of the intermediate hosts which, when consumed, cause infection of the shrew.

Both *S.araneus* and *S.minutus* have a very varied diet (Pernetta 1976a; Churchfield & Brown, 1987; Churchfield, 1990), consuming almost any suitably sized invertebrate available to them. Thus any invertebrate which might come into contact with infective stages (eggs or larvae) of shrew parasites, perhaps by feeding on organic matter in the soil, is a potential intermediate host for these helminths. There are relatively few publications on the subject of larval stages in intermediate hosts of shrew parasites when compared to the literature on the adult parasites in their definitive host. However, a large variety of intermediate hosts have been reported for some of the helminth species.

Not all helminth parasites require an intermediate host in order to complete their life cycle, some species may be directly transmitted between individuals of the definitive host species or may have free-living larval stages. However, because of the nature of the feeding habits of shrews, most, if not all of the helminths affecting *S.araneus* and *S.minutus* are likely to require an invertebrate intermediate host for successful transmission.

The cestode species recovered from the two shrew species belong either to the Dilepididae, of which all known life cycles are indirect (requiring an intermediate host) or to the Hymenolepididae, of which the rat tapeworm, *Hymenolepis nana*, is the only species known to have a direct life cycle (Lewis, 1987). Larvae of *Choanotaenia crassiscolex* (Dilepididae) have been found in representatives of several families of gastropod (Kisielewska, 1958a; Prokopic & Zdarska, 1958; Rawson & Rigby, 1960; Lewis 1968). Larvae of hymenolepid species whose

definitive hosts are *S.araneus* and *S.minutus* have been found in the following invertebrates: fleas (Siphonaptera) belonging to the genera, *Ctenopthalamus* (Prokopič, 1969) and *Palaeopsylla* (Smit 1974, 1978); beetles (Coleoptera) of the following families: Carabidae (Kisielewska, 1959), Catopidae (Kisielewska, 1958b), Geotrupidae (Kisielewska, 1959, 1960b, 1961; Prokopič & Karapcanski, 1973) Scarabeidae (Joyeux & Baer, 1936a; Kisielewska, 1959, 1960b, 1961; Rysavy & Prokopič, 1965; Prokopič, 1968a) and Staphylinidae (Prokopič, 1967); Orthoptera (Rysavy & Prokopič, 1965; Rysavy, 1989); in one species of isopod, *Glomeris limbata* (Joyeux & Baer, 1936a) and in one collembolan, *Tomocerus (Pogonognathellas) flavescens* Tullberg, 1871 (Prokopič, 1968b).

All known species of digeneans have indirect life cycles, usually involving two intermediate hosts. The digeneans recovered in the present study belonged to the following families: Brachylaemidae, Dicrocoeliidae and Plagiorchidae. Several species of gastropods have been identified as intermediate hosts of brachylaemids (Foster, 1957; Pojmanska, 1959, 1961; Lewis 1964, 1968, 1969). No information on dicrocoeliid life cycles was found in the literature. Plagiorchid life cycles usually involve a freshwater snail such as *Planorbis corneus* (L.) as the first intermediate host, with the second intermediate host being either a freshwater snail or the larva of a freshwater insect (Pojmanska, 1961; Bock, 1982). Pojmanska (1961) tried unsuccessfully to infect several species of terrestrial snail with *Opisthioglyphe sobolevi*.

Nematodes from the following six families were recovered in the present study: Acuariidae, Angiostrongylidae, Ascarididae, Heligmosomidae, Trichuridae (sub-family Capillariinae) and Strongyloididae. Intermediate hosts are not known for any of the nematode species recovered. The life cycles of many species of nematode are known to be direct, for example the heligmosomid nematode, *Heligmosomoides polygyrus*, a parasite of the wood mouse, *Apodemus sylvaticus* (L.) (Lewis, 1987). Transmission to the mouse is effected by the ingestion of vegetation contaminated with L3 larvae. Since the diet of *Sorex araneus* and *S.minutus* is almost entirely insectivorous, transmission is unlikely to occur in this way. Hence nematodes of the genera *Porrocaecum* (Ascarididae) and *Longistriata* (Heligmosomidae) are likely to require an invertebrate intermediate host, even

though they belong to families whose members are usually transmitted directly.

Moravec et al. (1987) reported that the life cycles of only 7% of the species of Capillariinae had been elucidated. Some species, such as *Calodium hepaticum*, a parasite of carnivores, rodents and primates, have a direct life cycle while others such as *Aonotheca erinacei*, a parasite of the hedgehog, *Erinaceus europaeus* L., and *Eucoleus aerophilus*, a parasite of carnivores, require an intermediate host (in both these cases an earthworm) for successful transmission.

Acuariid nematodes are known to utilise intermediate hosts since larvae of Acuariidae have been found in the isopod, *Hemilepistus pectinalus* by Sultan et al. (1980).

Larval stages of the angiostrongylid nematode, *Angiostrongylus malaysiensis*, a parasite of *Rattus spp.* which is closely related to *Stefanskostrongylus soricis*, have been found in several species of freshwater and land snail (Lim et al. 1977).

The intermediate host of the acanthocephalan *Gordiorhynchus aluconis* is not known, but Sultan et al. (1980) found the acanthor stage of *Gordiorhynchus lanseoides* (Petrotschenko, 1946) in the isopod, *Hemilepistus pectinalus*.

In the present study invertebrates were collected at intervals during a one-year period by means of pitfall traps, and examined for the presence of larval stages of helminth parasites. The records of the numbers of invertebrates collected also provided information on the seasonal variation in availability of intermediate hosts (Chapter 6).

Materials and Methods

Invertebrates were collected between January and November 1990 from the Silwood Park, Cranbourne Chase and Lipper Pond sites while trapping of shrews was being carried out.

Ten or twenty pitfall traps were used at each site. At Silwood Park five pitfall traps were placed on each of the four successional plots on which Longworth trapping was carried out, making a total of twenty pitfall traps on the site as a whole.

The pitfall traps consisted of plastic cups 7.0cm in diameter and 7.7cm in height which were sunk into the ground so that their rims were flush with the surface. A small amount of water was placed in the bottom of the cups to make it difficult for trapped invertebrates to escape.

Pitfall trapping was carried out over a period of three days and nights, even when a slightly shorter time period was used for Longworth trapping at Silwood Park. The traps were emptied each day during the three day trapping period. Any snails present on the surface of the Longworth traps were also collected.

The invertebrates obtained were stored in a refrigerator and examined while still fresh so that any parasitic stages present were still alive. The invertebrates were identified to species where possible and examined by gently crushing between two slides; the exoskeleton of larger coleopterans and gastropods was removed before examination.

Anything which looked like a parasite was carefully teased away from the invertebrate tissues and examined under high power. Those parasites recovered were drawn, photographed and identified where possible.

Results

Invertebrates Recovered

The species of invertebrates obtained from the pitfall traps are shown in Tables 5.1, 5.2, 5.3. All specimens of Coleoptera collected were examined for helminth parasites, but not all of the other invertebrates were examined as the probability of finding helminth larval stages in some of them was thought to be very low. The numbers of the non-coleopteran invertebrates which were actually examined are shown in Table 5.4. In addition to the invertebrates collected from the pitfall traps this table also includes 44 *Vitrina pellucida* (Muller), 4 *Oxychilus alliarius* and 1 *Cepaea nemoralis* (L.) found on or in the Longworth traps at Silwood during the February 1990 trapping session; 2 *Oxychilus alliarius* and 1 *O.helveticus* found in/on the Silwood Longworth traps in June 1990; 9 slugs obtained from Silwood in April 1990, and 6 slugs from Lipper Pond in January/February 1990.

Larval Helminths Recovered

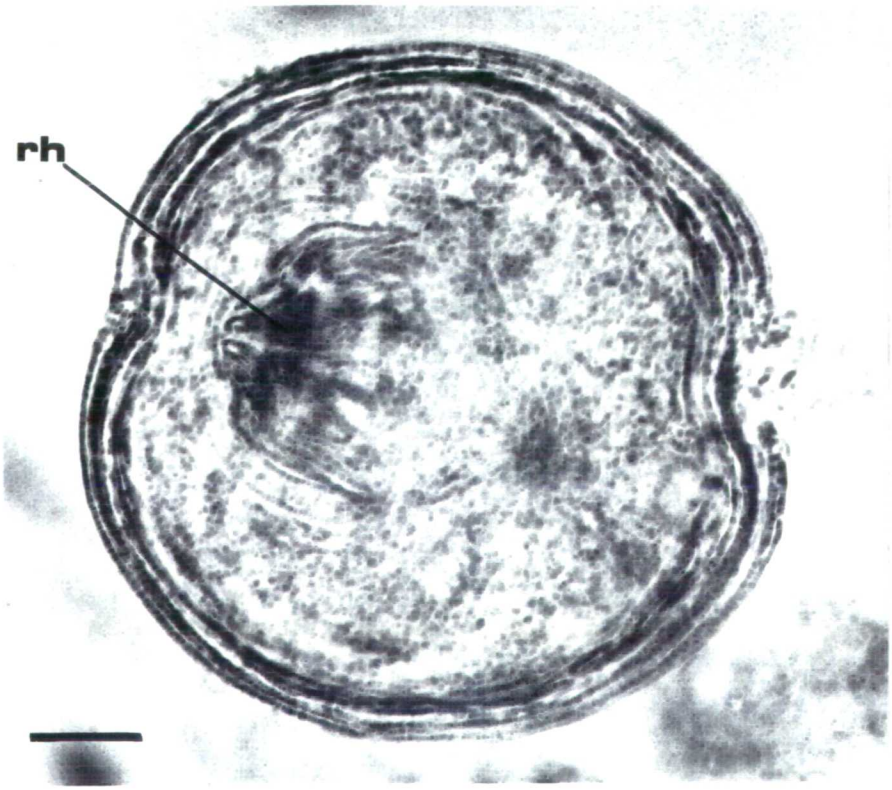
Cestodes

Cysticerci of *Choanotaenia crassiscolex* were recovered from 4 of 50 specimens of the gastropod snail, *Vitrina pellucida*. One specimen was found in each of the snails parasitised. The cysts containing the cestode larvae were 429-594μm in diameter. The cestode could be positively identified by the shape, size (57-59μm) and number (18-20) of hooks present.

1 of 2 adult *Anthobium unicolor* (Marsham, 1802) (Staphylinidae) was parasitised by approximately 20 cysticerci of *Hymenolepis schaldybini*. The cysts were spherical with a diameter of approximately 120μm. The cestode species could be positively identified by the ten hooks of characteristic shape and size (Plate 5.1).

1 of 1 adult *Pterostichus melanarius* (Illiger, 1798) (Carabidae) contained 4 cysts resembling those of the other two species of cestode found. They were roughly spherical with a diameter of 290-297μm. No recognisable structures could be seen inside them.

19 other specimens of *Pterostichus sp.* were found to be free of parasites.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Digeneans

Metacercariae of *Brachylaemus sp.*, probably *B.fulvus*, were recovered from 1 of 2 *Vitrina pellucida* and from 2 of 4 *Oxychilus helveticus*. One of the specimens of *O.helveticus* contained two metacercariae, the other infected gastropods contained single parasites. The metacercariae measured 2.32-2.85mm in length with a maximum width of 0.77-0.86mm. The oral sucker measured 244x251μm, the ventral sucker 281x284μm. The gonads were immature; the testes measured 92-119x92-106μm and the ovary 92x79μm.

Nematodes

Two types of nematode larvae were recovered from the invertebrates examined:

(1) Nematode larvae measuring 568-792μm long, with a maximum diameter of 25-30μm were recovered from 4 of 50 *Vitrina pellucida* and from 9 of 12 *Geotrupes sp.* (Coleoptera, Geotrupidae). Only one nematode was present in each of the gastropods examined, but the beetles each harboured several nematodes.

(2) Nematode larvae 950-1084μm long with a maximum diameter of 64μm were recovered from 1 of 27 *Philoscia muscorum*, 1 of 28 *Ophyiulus pilosus* and 8 of 35 *Glomeris marginata*. A single worm was present in the specimen of *P.muscorum* examined and 1-5 worms in the other invertebrates examined. The nematodes possessed a terminal cuticular spike and a convoluted oesophagus, reminiscent of one of the types of nematode larvae recovered from the liver of *S.araneus* (Chapter 3), however on closer examination it was found that the nematodes belonged to either the order Tylenchida and suborder Hexatylina, or possibly to the order Aphelenchida; since these taxa are not parasites of vertebrates the nematodes recovered from the invertebrates were not related to those larvae found in *S.araneus*.

Table 5.1. List of invertebrates collected at Cranbourne Chase, Windsor Great Park during 1990. 10 pitfall traps were used except during March when 20 were used.

Invertebrate type	Sampling dates				
	29/1-1/2/90	26-29/3/90	21-24/5/90	17-20/7/90	13-15/9/90
Carabidae	2	8	10	1	1
Staphylinidae	2	7	0	0	0
Curculionidae	0	0	0	2	0
Total Coleoptera (adults)	4	17	10	3	1
Coleoptera larvae	1	2	22	0	0
Diptera	0	0	0	3	0
Collembola	15	28	58	9	1
Isopoda	0	2	6	0	7
Chilopoda	1	0	0	0	0
Diplopoda	0	0	1	0	0
Araneae	1	11	78	32	8
Opiliones	1	8	3	7	1
Gastropoda	0	0	0	0	0
Acarina	0	0	0	160	0
Others	0	0	2	7	3

Table 5.2. Invertebrates collected at Lipper Pond, Windsor Great Park during 1990. 10 pitfall traps were used except during March when 20 were used.

Invertebrate type	Sampling dates				
	29/1-1/2/90	26-29/3/90	21-24/5/90	17-20/7/90	13-15/9/90
Carabidae	2	5	10	11	0
Staphylinidae	1	5	0	0	0
Curculionidae	0	0	20	0	0
Total Coleoptera	3	10	33	11	0
Coleoptera larvae	2	0	4	0	0
Diptera	3	0	12	8	6
Collembola	4	3	35	6	0
Isopoda	3	11	7	4	3
Chilopoda	0	0	0	0	0
Diplopoda	3	15	22	2	9
Araneae	19	27	44	30	42
Opiliones	3	5	6	6	6
Gastropoda	0	0	1	0	0
Acarina	0	0	13	34	3
Others	3	6	5	17	0

Table 5.3. Invertebrates collected at Silwood Park (20 pitfall traps).

Invertebrate type	Sampling dates					
	29/1-1/2/90	26-29/3/90	21-24/5/90	17-20/7/90	13-15/9/90	12-15/11/90
Carabidae	3	15	10	30	18	3
Staphylinidae	5	5	0	1	6	0
Curculionidae	0	8	2	2	0	6
Total Coleoptera	6	32	15	34	25	9
Coleoptera larvae	3	4	1	0	1	0
Diptera	1	4	5	3	5	2
Collembola	9	24	21	17	2	2
Isopoda	0	6	4	31	7	8
Chilopoda	0	0	0	0	1	1
Diplopoda	14	44	33	2	3	8
Araneae	20	65	19	64	34	11
Opiliones	1	9	18	22	4	3
Gastropoda	8	1	2	0	0	3
Acarina	0	0	2	41	0	0
Others	1	2	15	30	12	3

Table 5.4. Numbers of invertebrates (except Coleoptera) examined.

Invertebrate	Number examined
Diplopoda/Chilopoda	
Ophyiulus pilosus	28
Glomeris marginata	40
Cylindroiulus caeruleocinctus	24
Others	41
Gastropoda	
Vitrina pellucida	50
Oxychilus cellarius	6
Oxychilus helvaticus	4
Other snails	7
Slugs	18
Isopoda	
Philoscia muscorum	27
Others	7
Araneae	141
Collembola	79
Opiliones	28
Lepidoptera larvae	10
Diptera	9
Lumbricidae	4
Formicidae	1
Hemiptera	1

Discussion

A wide variety of invertebrates have been examined in the present investigation and although in many cases the numbers examined were too small to provide much information on the prevalence of infection, the present study constitutes an important addition to the current knowledge of the life cycles of some helminths.

A large number of spiders (Araneae) were examined and found to be free of infection. This is as expected since they would have had little opportunity of coming into contact with parasitic larvae.

Invertebrates may become infected by ingesting eggs or larvae of helminths in their food as in the case of infection of *Tribolium confusum* (Coleoptera) by *Hymenolepis diminuta* (Keymer & Anderson, 1979) or by direct penetration by the larval stages as occurs in the infection of *Lymnaea spp.* by the liver fluke, *Fasciola hepatica* (Whitfield, 1982).

Spiders feed on the body fluids of other invertebrates and are therefore unlikely to come into contact with larval stages through their food. Their relatively fast movement over the ground surface and occupation of a web when stationary renders them inaccessible to larval stages which actively penetrate their host.

The 79 Collembola examined were also found to be uninfected. Their habit of feeding on organic matter in the soil would facilitate the ingestion of helminth eggs which had been passed out in the faeces of shrews. However, the only record of helminth larvae being found in Collembola is by Prokopic (1968b) who found *Hymenolepis spinulosa* in 4 of 22 specimens of *Tomocerus flavescens*. *H.spinulosa* was not found in the present study. Although Collembola may have a high probability of ingesting helminth larval stages, their effectiveness as intermediate hosts may be limited by their scarcity in the diet of shrews (Churchfield, 1982, 1984; Churchfield & Brown, 1987; Churchfield, Hollier & Brown, 1991).

Cestode Larvae

The cysticerci of *Choanotaenia crassiscolex* recovered in the present study could be identified by the size, shape and number of the rostellar hooks. The size of the cysts was also similar to the size of those found by Harper (1930), Kisielewska (1958a) and Rawson & Rigby (1960). The latter authors gave a detailed description of the cysticerci.

Larval stages of *C.crassiscolex* have been found by Kisielewska (1958a, 1961) in eleven species of snail, in a further two species by Rawson & Rigby (1960) and Lewis (1969). In the present study only *Vitrina pellucida* was examined in significant numbers. 8% were found to be infected compared to 69% by Kisielewska (1958a).

Gastropods are known to feature significantly in the diet of *S.araneus*, but are less important in the diet of *S.minutus* (Pernetta 1976a; Churchfield, 1984; Churchfield & Brown, 1987; Churchfield, Hollier & Brown, 1991). This may partially explain the much greater prevalence and intensity of infection in *S.araneus*. During the course of the present study fragments of the shells of *V.pellucida* were sometimes observed in Longworth traps occupied by *S.araneus*. Hence *V.pellucida* is an effective intermediate host of *C.crassiscolex*.

The cysticerci of *Hymenolepis schaldybini* found in the staphylinid beetle, *Anthobium unicolor* in the present study were very similar to those found by Kisielewska (1958b) in *Catops sp.* and by Prokopic (1968a) in *Oeceoptoma thoracica*. Both authors classified the cysticerci as *Neoskrjabinolepis singularis*, but from the measurements which they gave for the rostellar hooks (36-42μm and 32-34μm respectively) it is clear that they in fact found *Hymenolepis schaldybini*. The taxonomy of this species has been discussed in Chapter 3. *A.unicolor* is a new intermediate host for *H.schaldybini*.

A.unicolor is a carnivorous beetle preying on small invertebrates such as Collembola. As discussed above, cysticerci have been found in Collembola so it is possible that the beetle may become infected through its prey. Both *S.araneus* and *S.minutus* are known to feed on staphylinid beetles of similar size (3-4mm) to *A.unicolor* (Pernetta, 1976a; Churchfield, 1990) so this beetle is likely to be an important intermediate host for *H.schaldybini*.

The unidentified cysts obtained from *Pterostichus melanarius* appeared to be the cysticerci of a cestode species. It is likely that any rostellar hooks present would have been easily visible, so the cysticerci may have been those of *Hymenolepis diaphana* which has an unarmed rostellum. Their diameter was within the range found by Kisielewska (1960b) for *H.diaphana* in *Geotrupes stercorosus* Panz. *H.diaphana* has not previously been recorded in *Pterostichus sp.*

Digenean Larvae

The arrangement of the genitalia and the excretory system of the brachylaemid metacercariae found in the present study was very similar to that found by Lewis (1969) in metacercariae from *Zonitoides excavatus* and in adult specimens of *Brachylaemus fulvus* in both studies. Lewis (1968) demonstrated by successfully infecting non-parasitised *S.araneus* and laboratory mice that the metacercarial stages did in fact belong to the species, *Brachylaemus fulvus.*

Metacercariae of *B.fulvus* have also been found in *Zonitoides nitidus* (Muller) and *Goniodiscus rotundatus* (Muller) (Pojmanska, 1959) and in *Oxychilus orientalis* (Cless.), *Aegopinella epipedostoma* (Pagot), *Euconulus fulvus* (Mull.) and *Deroceras sp.* (Pojmanska, 1961).

The intermediate hosts found in the present study, *Vitrina pellucida* and *Oxychilus helveticus* have not previously been identified as hosts of *B.fulvus.*

Nematode Larvae

Larvae of two species of nematode were found in the present study, but neither type appears to be a parasite of shrews.

Other Possible Intermediate Hosts

A comparison of the prevalences of *Eucoleus oesophagicola, Liniscus incrassatus* and *Porrocaecum sp.* in *Sorex araneus* and *S.minutus* (Chapter 6) suggests that the intermediate host(s) of these species is an invertebrate eaten in much greater quantities by *S.araneus* than by *S.minutus.* An obvious candidate is a species of earthworm (Lumbricidae) since there only three records of *S.minutus* eating earthworms (Crowcroft, 1957; Churchfield & Brown, 1987, Churchfield, Hollier &

Brown, 1991) and earthworms have been recorded as intermediate hosts of capillariinids (Romashov, 1980; Moravec et al., 1987). In the present study an insufficient number of Lumbricidae was examined to reach any conclusions about their possible role as intermediate hosts of shrew parasites.

In addition to improving the current state of knowledge of the identity of some intermediate hosts of shrew parasites, the results obtained from pitfall trapping provided information on the seasonal variation in availability of intermediate hosts. Since a standardised sampling procedure was used throughout the year, the results obtained may be used to provide a quantitative measurement of the seasonality of abundance of each type of invertebrate.

The importance of the seasonal changes in availability of intermediate hosts will be discussed in Chapter 6.

Summary

(1) A large variety of invertebrates have been examined for the presence of parasite larvae.

(2) Cysticerci of *Choanotaenia crassiscolex* have been found in *Vitrina pellucida*, a known prey item of *S.araneus*.

(3) Cysticerci of *Hymenolepis schaldybini* have been found in *Anthobium unicolor* which is a new intermediate host for this species.

(4) Unidentified cysticerci, possibly those of *H.diaphana*, have been recovered from *Pterostichus melanarius*.

(5) Metacercariae of *Brachylaemus fulvus* have been found in *Vitrina pellucida* and *Oxychilus helveticus* which are new intermediate hosts for this helminth.

(6) The mode of transmission of the above helminths has been discussed.

(7) Further work on the life cycles of helminth parasites in shrews is required.

Chapter 6

Ecology of Helminth Parasites in *Sorex araneus* and *S.minutus* from Selected Areas in Southeast England.

Introduction

In order to gain an understanding of the possible effects of helminth parasites on the population dynamics of shrews, it is necessary to investigate both the temporal and spatial distribution of the parasites in their hosts.

Data on the prevalence of infection (percentage of hosts infected) provide information on the proportion of the host population upon which a given helminth species might be able to exert a regulatory affect, while the intensity value indicates the severity of the infection. The intensity indicates the mean number of parasites per infected host, but does not show how the parasites are distributed within the infected population. For example, a parasite may be fairly evenly distributed throughout the population so that no individual has a particularly large worm burden, alternatively a few hosts may have very large worm burdens while the remainder of the population are relatively unaffected. The pathogenic effects produced by parasites are generally related to the number of parasites present in the host (Anderson & May, 1978). Thus it is important to determine the distribution of the parasites within the host population.

The distribution of parasites in a population is generally overdispersed (Anderson & Gordon, 1982; Keymer, 1982). This uneven distribution might be the result of variation in levels of infection in hosts of different age (Kisielewska, 1961) or sex (Solomon, 1969) or may be due to genetic variation between individuals, perhaps affecting their immunological responses (Wakelin, 1985, 1987).

Prevalences and intensities might also vary seasonally and this may be due to changes in the availability of intermediate hosts (Kisielewska, 1961). A parasite might therefore exert a significant effect on the host population at a particular time of year while at other times it may have relatively little effect.

Having gained an insight into the temporal and spatial distribution of the parasite species present in a host population, it is then necessary to gauge the impact on individual hosts of the levels of infection encountered in the host population. In this way it is possible to assess the likelihood of a helminth species or group of species having a regulatory affect on the host population.

The success of a particular parasite is dependent on various factors concerned with the environment in which it lives, these include the presence of other helminths in the host, the presence of other definitive hosts and the suitability of the habitat for parasitic transmission.

Holmes (1961) showed that individual parasites were smaller in concurrent infections of rats with *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocephala) than in single-species infections. The distribution of the parasites in the intestine was also altered when both species were present in the same host. In the present study interspecific competition between helminth parasites was investigated by examining the distribution of helminths in the intestine.

It has been shown (Prokopic, 1959, 1970a, 1970b; Pojmonska, 1961; Prokopic et al., 1974) that the composition of the helminth fauna present in *S.araneus* and *S.minutus* is related to the habitat or biotope. This may be the result of differences in the suitability of the habitat for the intermediate hosts used by the helminths or for any free-living stages present in the life cycle. Thus differences in helminth fauna related to habitat may provide important information on the means of transmission employed by the parasite species concerned.

Information on the life cycles of helminth parasites may also be obtained by comparing the helminth faunas of closely related host species. Differences between host species may be due to host specificity of the helminths concerned, or may result from ecological differences such as differences in diet, which may cause the intermediate host(s) of a parasite species to be consumed by one potential definitive host species, but not by another (Kisielewska, 1961). If the latter is the case, comparison of the helminth faunas of two host species coupled with knowledge of their diet may lead to the discovery of the intermediate hosts involved. Ability of a parasite to infect more than one host may also increase its chances of survival. For example, in the Commonwealth of Independent States gerbils (*Meriones sp.* and *Rhombomys spp.*) act as reservoir hosts for human leishmaniasis. (Schad, 1982).

Many factors must therefore be taken into account when considering the impact of parasites on host populations. In the present study, the following areas have been investigated: Prevalence and intensity levels, the effect of host age and

sex, seasonal variation in infection levels, distribution of the parasites within the host population, distribution of the parasites within individual hosts, pathogenic effects, the effect of habitat, and the difference between the helminth faunas of the two host species.

This chapter describes the results of the above investigations on the helminths of *S.araneus* and *S.minutus*, with additional information on the ectoparasites present in these shrew species.

Materials and Methods

The study sites, sampling procedures and methods used in the examination of shrews for parasites have been described in Chapters 2-5. Other methodology used in this chapter is described under the relevant sub-headings. Shrew autopsy data was analysed using an "Open Access" database (Software Products International). Intensities of helminth infection in different samples of shrews were compared using the Mann-Whitney U test; only infected individuals were used in this calculation. The difference in the prevalence of infection between two samples was compared using the following means. A 2 x 2 contingency table was drawn up as shown below

	No. infected	No. uninfected
Sample 1	a	b
Sample 2	c	d

The expected values of a, b, c, d were then calculated by simple proportions and χ^2 calculated using the formula:

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Statistical tables were use to determine the significance of the value of χ^2.

Results

Helminth Parasites in the Common Shrew, *Sorex araneus*

(1) Overall Prevalences and Intensities of Infection

A total of 109 common shrews from Southeast England were examined thoroughly (Chapter 3): 45 from Cranbourne Chase, 37 from Lipper Pond and 21 from Silwood Park (some of these were accidental casualties found dead in the traps on the successional plots, while the majority were trapped on nearby grassland). The remaining six specimens were obtained from the following locations: three from Upper Norton, Oxfordshire (Ordnance Survey Grid Reference SP 310290), one from Chesham, Buckinghamshire and two from the grounds of "Alderhurst", part of the R.H.B.N.C. Biology Department.

Further helminth material was obtained from four shrews trapped at "Alderhurst" and four specimens trapped at "Huntersdale" (also part of the R.H.B.N.C. Biology Department) and twelve specimens trapped at Dungeness on the Kent Coast (Ordnance Survey Grid reference TR 074182). The latter 20 shrews were not examined thoroughly, due to insufficient time being available, so the results were not included in the database used for the calculation of overall prevalences and intensities and other quantitative results. These specimens were useful, however, in augmenting the species list of helminths found in British shrews.

Only one of the 109 *Sorex araneus* examined was not infected with helminth parasites, this was an immature specimen trapped in mid-June 1990 which would have had little time to become infected. Two other *S.araneus* had no cestode parasites, but all of the others were infected with nematodes.

The overall prevalences and intensities of the helminths from the sample of 109 shrews (Tables 6.1, 6.1a, 6.1b) indicate that the most abundant parasites comprised the cestodes *Choanotaenia crassiscolex* (prevalence 71%, intensity 11.0), *Hymenolepis schaldybini* (61%, 30.1) and *H.scutigera* (58%, 26.7); the digenean *Brachylaemus fulvus* (62%, 4.3) and the nematodes *Porrocaecum sp.* (74%, 13.0) and *Longistriata spp.* (81%, 19.6) especially *L.didas* (59%, 17.6).

Table 6.1. Prevalences and intensities of helminth parasites in *Sorex araneus* from various parts of Southeast England.

Helminth	Prevalence	Intensity
Choanotaenia crassiscolex	71%	11.0
C.hepatica	1%	22.0
Hymenolepis diaphana	15%	56.7
H.furcata	23%	4.8
H.infirma	8%	157.4
H.jacutensis	4%	9.8
H.prolifer	1%	89.0
H.schaldybini	61%	30.1
H.scutigera	58%	26.7
H.singularis	17%	8.9
All Hymenolepids	93%	58.0
Brachylaemus fulvus	62%	4.3
Dicrocoelium soricis	6%	2.3
Eucoleus oesophagicola	62%	3.1
E.kutori	6%	5.5
Liniscus incrassatus	41%	4.8
Parastrongyloides winchesi	49%	15.8
Porrocaecum sp.	74%	13.0
Stammerinema soricis	2%	1.0
Longistriata depressa	41%	5.7
L.didas	59%	17.6
L.thomasi	3%	1.0
L.trus	5%	2.4
All *Longistriata spp.*	81%	19.6
Nematode larva (a)	8%	5.6
Nematode larva (b)	5%	2.6
Gordiorhynchus aluconis	33%	5.5

An additional three helminth species were found in the sample of 20 shrews from Dungeness and the grounds of the R.H.B.N.C. Biology Department. *Opisthioglyphe sobolevi* (Digenea) was found only at Dungeness with a prevalence of 50% and worm burdens greater than 100 specimens in some cases. *Calodium cholidicola* (Nematoda) was found once at "Huntersdale" and *Prosorhynchus sp.* (Acanthocephala) was found once at "Alderhurst".

68 of the shrews were examined for ectoparasites, the others were not examined as they were found dead in the traps and ectoparasites are known to leave their hosts soon after its death (Smit, 1957); 42% were parasitised by fleas (intensity 2.7), 15% by ticks (intensity 2.6) and 6% by mites.

(2) Infection Levels Relative to Host Age

Between the beginning of June and the end of September (in both years), both adult and juvenile *S.araneus* were present at the same time thus the two generations could be compared without the differences being masked by any seasonal variation in infection.

Cestodes

Prevalences of infection were similar in both mature and immature shrews, although prevalences were slightly higher in juveniles for seven out of the ten cestode species. Intensity of infection was greater in juveniles than in adult specimens for 7 of the cestode species and was significantly larger in *H.schaldybini* (I_{juv}[1]=57.7, I_{ad}[2]=9.3, $0.001<P<0.01$) and *H.scutigera* (I_{juv}=52.7, I_{ad}=12.8, $0.001<P<0.01$).

[1] I_{juv} = Intensity of infection in the juvenile shrews

[2] I_{ad} = Intensity of infection in the adults

Nematodes

Prevalences and intensities were generally greater in adults than in juveniles. The differences were especially prominent in the case of *Porrocaecum sp.*, 87% of the adults being infected with a mean of 31.5 worms per infected host, while only 36% of the juveniles were infected with a mean worm burden of 2.1. These differences were statistically significant, for the prevalence values $0.001<P<0.01$ and for the intensity values $P<0.001$.

Prevalences and intensities of infection by the capillariinids *Eucoleus oesophagicola* and *Liniscus incrassatus* were significantly greater in adult than in juvenile shrews and the intensity of infection by *Longistriata spp.* was 35.2 in mature shrews compared with 10.9 in immature specimens ($0.001<P<0.01$).

Digeneans and Acanthocephalans

Prevalences and intensities of infection by digeneans were similar in both age groups. 27% of adult shrews were infected with the acanthocephalan *Gordiorhynchus aluconis* compared to 0% of the juveniles, but the intensity of infection was only 1.0.

Ectoparasites

Fleas were more abundant in adults (prevalence 83%, intensity 4.0) than in juveniles (57%, 2.5), while the reverse was true for ticks - no adults were infected compared with a 21% prevalence of infection in juveniles (intensity 2.7). Mites were not very abundant in either group. These differences were not statistically significant.

(3) Infection Levels Relative to Host Sex (In Mature Shrews)

Cestodes

The prevalence and intensity of *Choanotaenia crassiscolex* was twice as large in male as in female shrews, the difference in intensity values was not statistically significant, but there was a significant difference in the prevalence values

($0.01<P<0.05$). In general, prevalences and intensities were slightly higher in males than in females although the intensity of *Hymenolepis furcata* in females (23.0) was much higher than in males (1.7), this can be attributed to one female having 63 specimens of *H.furcata* which seemed to have been recently ingested due to their small size.

The mean number of cestodes per male shrew was 27.9 and the mean number per female was 27.2; this difference was not statistically significant.

Nematodes

Overall there were more nematodes in female than in male shrews (66.3 per female and 51.7 per male - not statistically significant) although the only really marked difference between the two was the greater abundance of *Parastrongyloides winchesi* in females where the prevalence was 47% and the intensity 33.9 compared with 35% and 8.5; these differences were not statistically significant.

Digeneans and Acanthocephalans

Digeneans and acanthocephalans were slightly more abundant in male than in female shrews.

Ectoparasites

The number of mites and ticks present was low in both sexes. More female than male shrews were infected with fleas (87% compared to 67%) but the intensity of infection was greater in the males (4.2 compared to 2.9). These differences were not statistically significant.

Helminth Parasites in the Pygmy Shrew, *Sorex minutus*

(1) Overall Prevalences and Intensities of Infection

A total of 72 pygmy shrews were examined thoroughly: 47 from Cranbourne Chase, 23 from Lipper Pond and 2 from Silwood Park. Helminth material was also obtained from five specimens trapped at "Alderhurst". Three of the latter were infected with the only specimens of *Stammerinema soricis* recovered from *S.minutus* in the present study.

Only one individual, a juvenile trapped at Lipper Pond, was free of helminth parasites; this shrew was parasitised by two ticks.

The overall prevalences and intensities of parasites from the 72 *S.minutus* (Tables 6.2, 6.2a, 6.2b) indicate that the most abundant parasites were the hymenolepids *Hymenolepis schaldybini* (prevalence 68%, intensity 24.5) and *H.furcata* (39%, 3.8); the digenean *Dicrocoelium soricis* (21%, 3.2), the nematodes *Stefanskostrongylus soricis* (50%, 6.8) and *Longistriata spp.* (83%, 8.6) especially *L.didas* (56%, 7.2) and the acanthocephalan *Gordiorhynchus aluconis* (46%, 11.5).

Fifty-seven of the pygmy shrews were examined for the presence of ectoparasites; 21% were infected with fleas (intensity 1.4), 60% with ticks (intensity 5.5) and 9% with mites.

(2) Infection Levels Relative to Host Age

Cestodes

Prevalences and intensities of infection were similar in both mature and immature shrews and none of the differences were statistically significant.

Nematodes

With the exception of *Parastrongyloides winchesi* and nematode larva (a), nematodes were more prevalent in adult than in juvenile shrews. The intensity of infection by all nematode species was higher in adults than in juveniles. Sample

Table 6.2. Prevalences and intensities of helminth parasites in 72 *Sorex minutus* from Berkshire.

Helminth	Prevalence	Intensity
Choanotaenia crassiscolex	4%	1.0
Hymenolepis diaphana	8%	26.0
H.furcata	39%	3.8
H.infirma	8%	24.3
H.jacutensis	4%	7.0
H.schaldybini	68%	24.5
H.scutigera	13%	6.8
H.singularis	10%	5.6
All Hymenolepids	85%	28.3
Brachylaemus fulvus	8%	2.8
Dicrocoelium soricis	21%	3.2
Eucoleus oesophagicola	1%	1.0
E.kutori	32%	4.3
Liniscus incrassatus	1%	1.0
Parastrongyloides winchesi	32%	2.8
Porrocaecum sp.	13%	1.4
Stefanskostrongylus soricis	50%	6.8
Longistriata depressa	22%	2.2
L.didas	56%	7.2
L.thomasi	4%	1.3
L.trus	26%	1.6
All *Longistriata spp.*	83%	8.6
Nematode larva (a)	18%	3.0
Gordiorhynchus aluconis	46%	11.5

sizes were generally too small to allow statistical tests to be carried out, but the intensity of *Stefanskostrongylus soricis* was significantly greater in mature specimens ($0.01<P<0.05$).

Digeneans and Acanthocephalans

No specimens of *Brachylaemus fulvus* were present in either age group, but *Dicrocoelium soricis* was more abundant in adult hosts. *Gordiorhynchus aluconis* was also more abundant in adults. Neither of these differences were statistically significant.

Ectoparasites

The prevalence and intensity of fleas was similar in both adults and juveniles. Mites and ticks were slightly more abundant in adults, but these differences were not statistically significant.

(3) Infection Levels Relative to Host Sex (Mature Shrews)

There was no obvious pattern of differences in infection of male and female shrews by helminths or ectoparasites. None of the observed differences in intensity were statistically significant and there was only one significant difference in prevalence - the prevalence of *Hymenolepis furcata* in males (58%) was significantly greater ($0.01<P<0.05$) than in females (26%).

Comparison of Infection Levels in *Sorex araneus* and *S.minutus*

In general, prevalences and intensities of helminths were higher in *S.araneus* than in *S.minutus*.

Cestodes

Appreciable differences existed in the case of the following: *Choanotaenia crassiscolex* (P_{sa}[1] = 71%, P_{sm}[2] = 4%, P<0.001; I_{sa}[3] = 11.0, I_{sm}[4] = 1.0); *Hymenolepis scutigera* (P_{sa} = 58%, P_{sm} = 13%, P<0.001; I_{sa} = 26.7, I_{sm} = 6.8). *C.hepatica* and *H.prolifer* were absent from *S.minutus*, but present in low numbers in *S.araneus*.

Nematodes

Prevalences and intensities of infection were generally greater in *S.araneus* than in *S.minutus* especially in the case of *Porrocaecum sp.* (P<0.001), *Eucoleus oesophagicola* (P<0.001), *Liniscus incrassatus* (P<0.001) and *Parastrongyloides winchesi* (0.01<P<0.05). Exceptions to this rule were the capillariinid *Eucoleus kutori* which is much more prevalent in *S.minutus* (P<0.001) and the angiostrongylid, *Stefanskostrongylus soricis* which was not found in *S.araneus*.

Digeneans and Acanthocephalans

The prevalence (62%) and intensity (4.3) of *Brachylaemus fulvus* was much greater (for the prevalence value P<0.001) in *S.araneus* than in *S.minutus* (prevalence 8%, intensity 2.8) while the reverse was true for *D.soricis* (P_{sa} = 6%, P_{sm} = 21%, P<0.001; I_{sa} = 2.3, I_{sm} = 3.2, not statistically significant).

Gordiorhynchus aluconis was more abundant in *S.minutus* than in *S.araneus* although the differences in prevalence and intensity were not statistically

---

[1] P_{sa} = Prevalence of infection in *Sorex araneus*

[2] P_{sm} = Prevalence of infection in *S.minutus*

[3] I_{sa} = Intensity of infection in *S.araneus*

[4] I_{sm} = Intensity in *S.minutus*

significant.

Ectoparasites

Ticks were more abundant on *S.minutus* (P_{sa} = 15%, P_{sm} = 60%, P<0.001; I_{sa} = 2.6, I_{sm} = 5.5, not statistically significant) while fleas were more common on *S.araneus* (P_{sa} = 62%, P_{sm} = 21%, P<0.001; I_{sa} = 2.7, I_{sm} = 1.4, not statistically significant).

Seasonal Variation in Infection Levels in Both Host Species

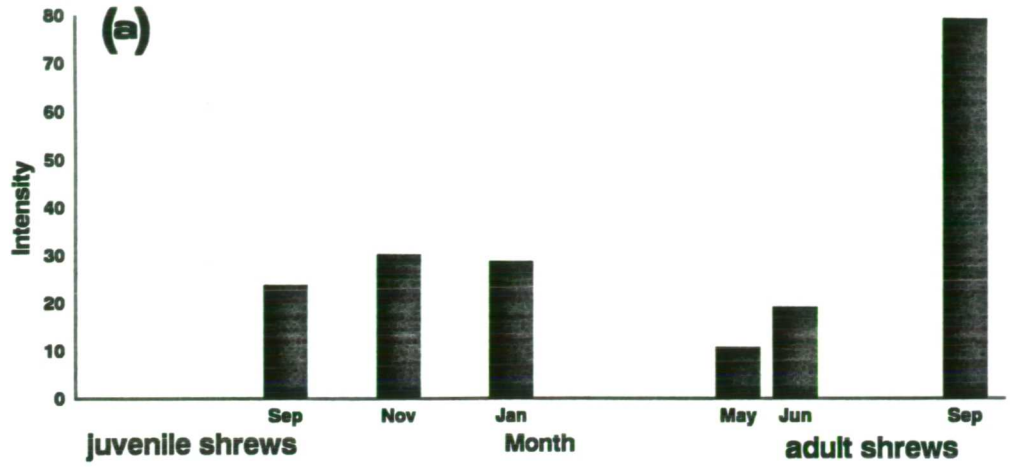
Cestodes

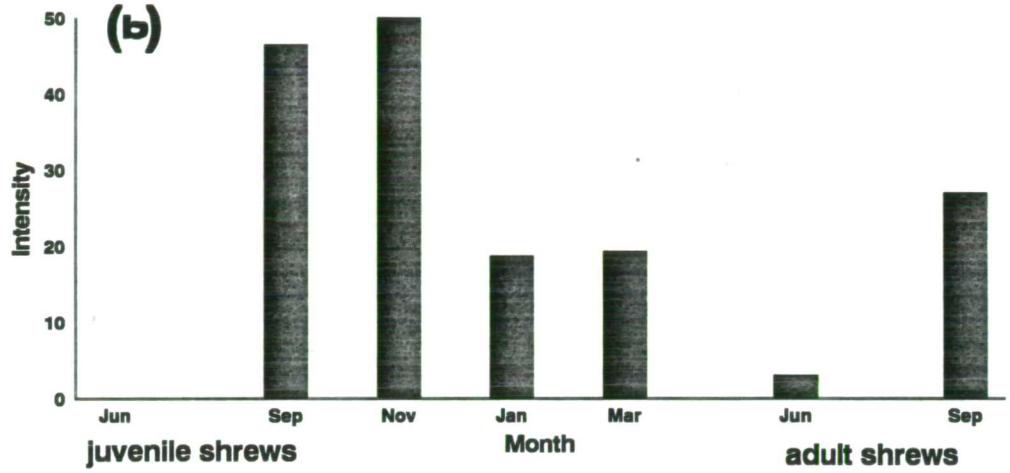
The hymenolepid cestodes showed definite seasonal trends in prevalence and intensity. Juveniles shrews rapidly became infected with high worm burdens in the summer months, prevalences and intensities dropped markedly in the autumn, remained low during the winter, but increased rapidly in the late spring and summer. This was especially obvious in the two most abundant species *Hymenolepis schaldybini* (Figure 6.1) and *H.scutigera* (Figure 6.2) where there was some similarity between the graphs for intensity of infection by the parasites and abundance of their intermediate hosts (carabid beetles in the case of *H.schaldybini* and fleas in the case of *H.scutigera*).

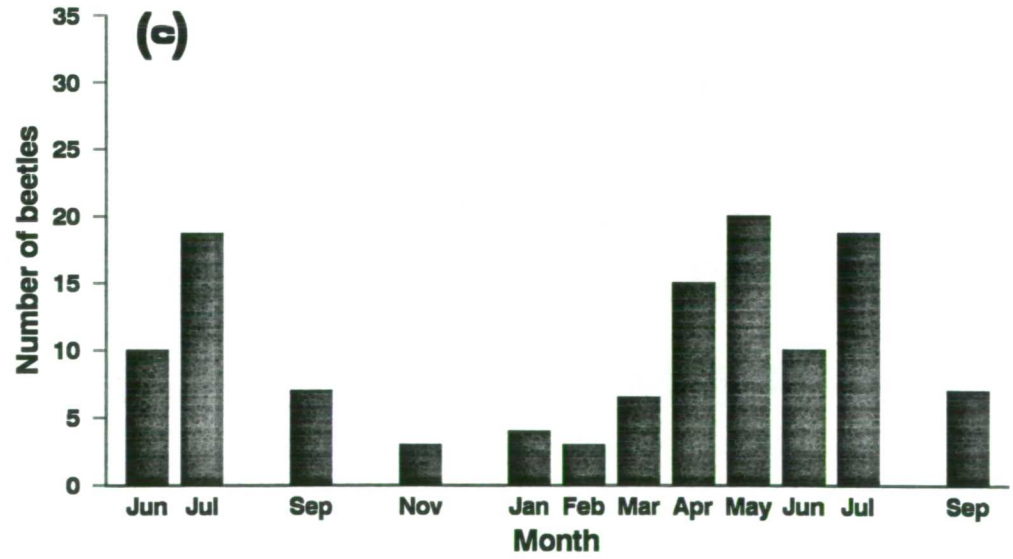
Seasonal trends are not obvious in *Choanotaenia crassiscolex*, but prevalences and intensities of this parasite were certainly higher in the autumn/winter than was the case for the hymenolepid species.

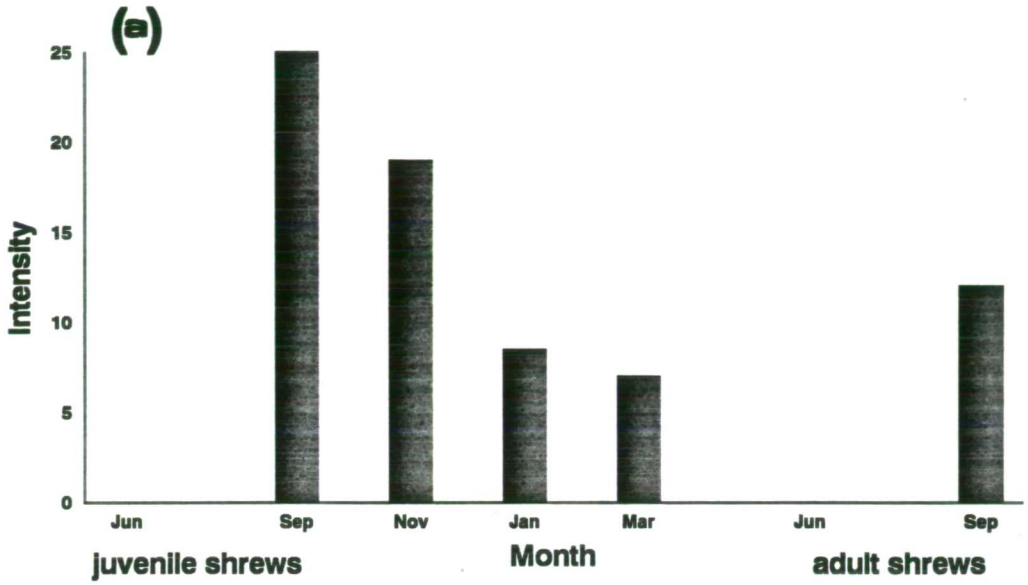
Nematodes

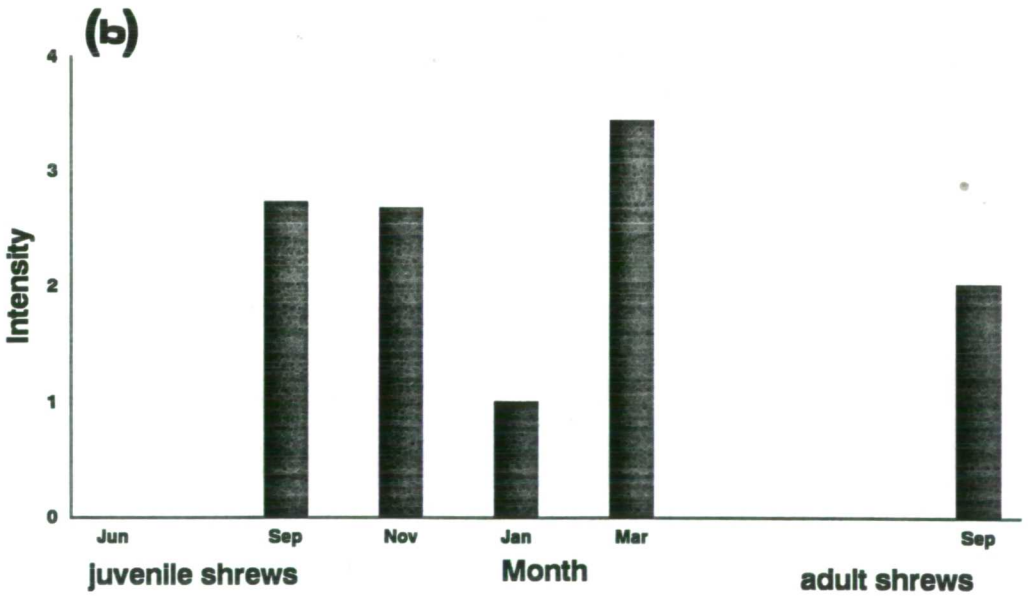
Abundance of *Porrocaecum sp.* increased at a fairly uniform rate throughout the year. Most of the other nematodes appeared to show a similar trend to that observed in the hymenolepid cestodes, *Longistriata spp.* in both shrew species (Figure 6.3) and *Parastrongyloides winchesi* in *S.araneus* showing a particularly marked increase in intensity in the summer.

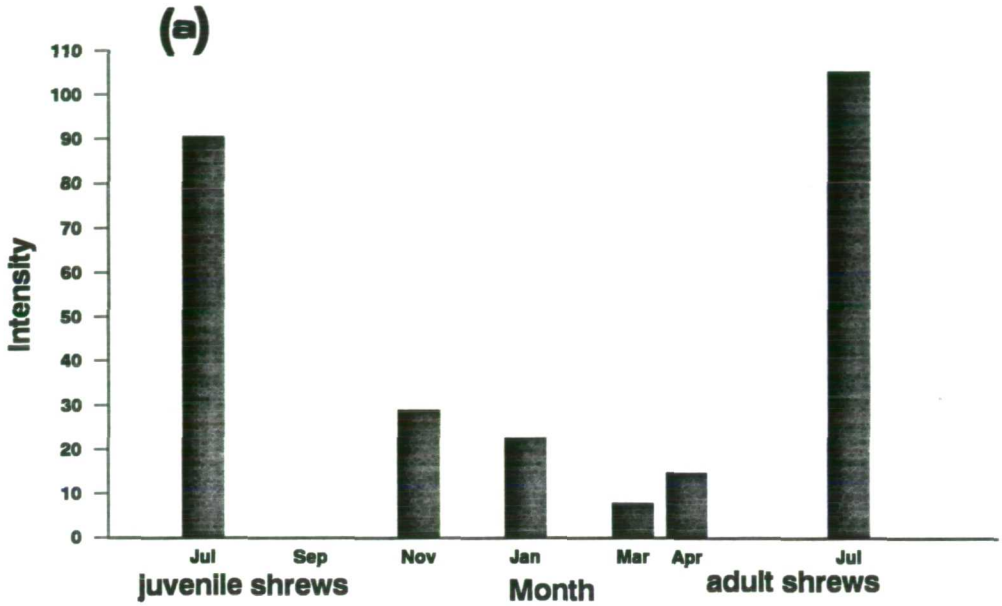

(a)
Intensity
80
70
60
50
40
30
20
10
0
Sep
Nov
Jan
May
Jun
Sep
juvenile shrews
Month
adult shrews

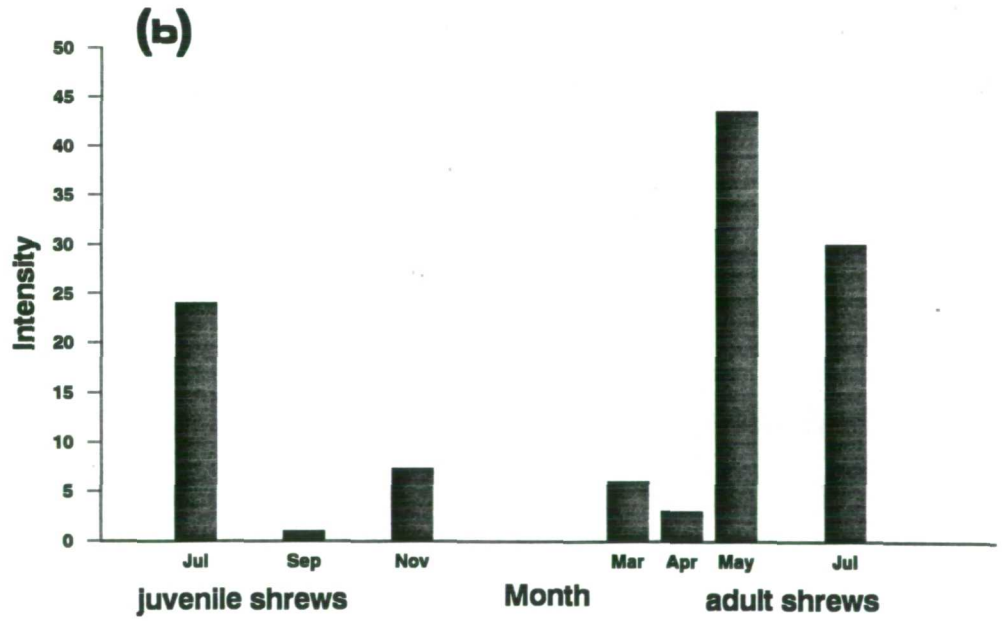

(b)
Intensity
50
40
30
20
10
0
Jun
Sep
Nov
Jan
Mar
Jun
Sep
juvenile shrews
Month
adult shrews

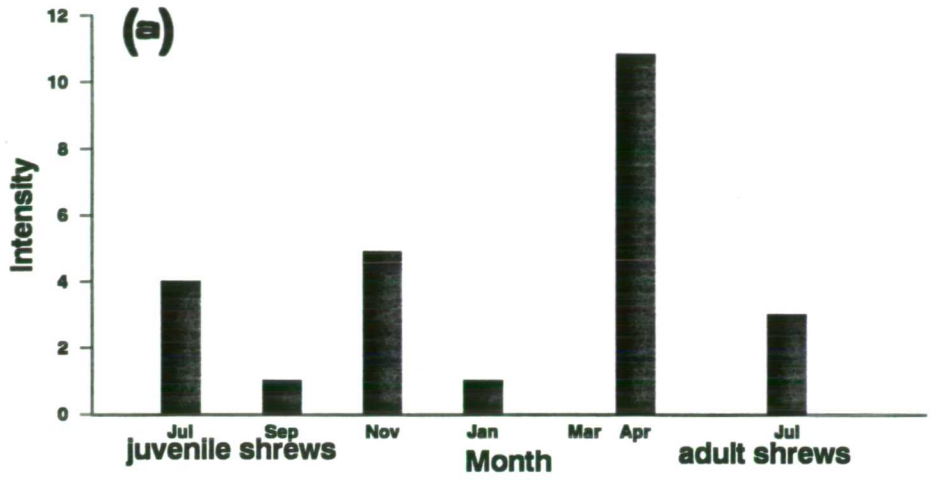

(c)
Number of beetles
35
30
25
20
15
10
5
0
Jun
Jul
Sep
Nov
Jan
Feb
Mar
Apr
May
Jun
Jul
Sep
Month

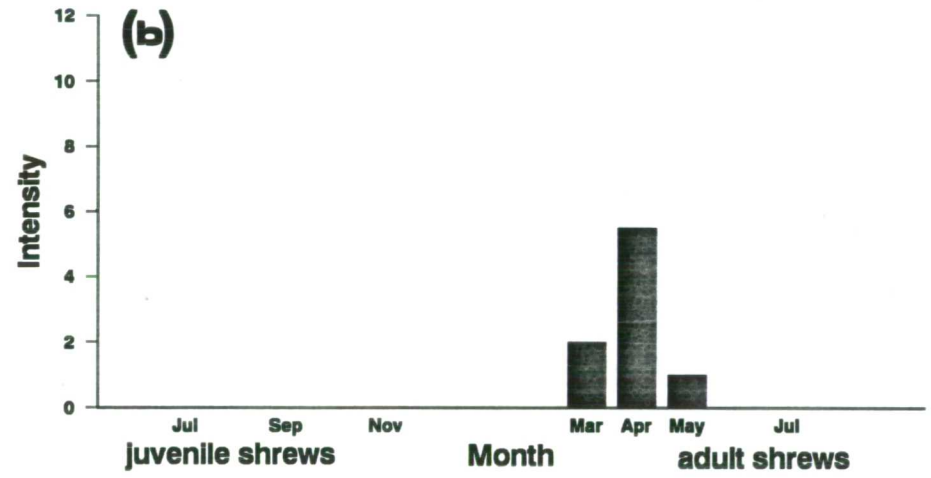

(a)
Intensity
25
20
15
10
5
0
Jun
Sep
Nov
Jan
Mar
Jun
Sep
juvenile shrews
Month
adult shrews

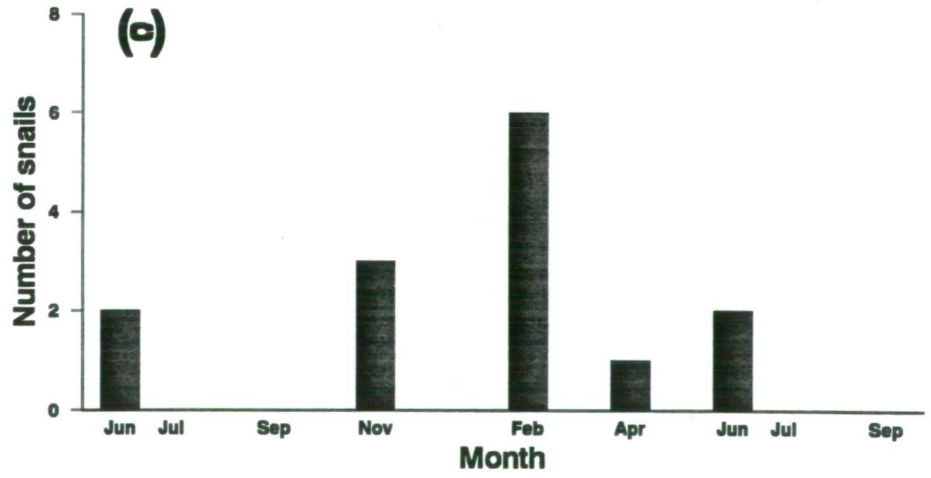

(b)
Intensity
4
3
2
1
0
Jun
Sep
Nov
Jan
Mar
Sep
juvenile shrews
Month
adult shrews


(a)
Intensity
110
100
90
80
70
60
50
40
30
20
10
0
Jul
Sep
Nov
Jan
Mar
Apr
Jul
juvenile shrews
Month
adult shrews


(b)
Intensity
50
45
40
35
30
25
20
15
10
5
0
Jul
Sep
Nov
Mar
Apr
May
Jul
juvenile shrews
Month
adult shrews


(a)
Intensity
12
10
8
6
4
2
0
Jul
Sep
Nov
Jan
Mar
Apr
Jul
juvenile shrews
Month
adult shrews


(b)
Intensity
12
10
8
6
4
2
0
Jul
Sep
Nov
Mar
Apr
May
Jul
juvenile shrews
Month
adult shrews


(c)
Number of snails
8
6
4
2
0
Jun
Jul
Sep
Nov
Feb
Apr
Jun
Jul
Sep
Month

Digeneans and Acanthocephalans

Brachylaemus fulvus was more abundant in the spring (March-May) than at other times of the year and this peak in intensity closely followed the peak in snail abundance (Figure 6.4). The scarcity of *Dicrocoelium soricis* made it difficult to elucidate any seasonal trends, but this species also appeared to be more abundant in the spring. Prevalences and intensities of *Gordiorhynchus aluconis* showed no obvious trends, but certainly did not increase as the shrews aged as was the case with infections of *Porrocaecum sp.*

Ectoparasites

Ectoparasites were much more abundant during the summer than during the rest of the year.

Seasonal Variation in Faecal Egg Counts

Seasonal variation in infection at Silwood Park was monitored by the examination of the faecal samples (Chapter 4) of five *S.araneus* at regular intervals from July 1989 to June 1990.

Tables 6.3 and 6.4 show the presence (✓) or absence (x) of the more common helminth parasites in each shrew during each sampling period. Two faecal samples were examined where possible, but the figure '1' signifies that only one sample was examined and a '-' denotes that no samples were examined.

Cestodes

The prevalence of *Choanotaenia crassiscolex* was 100% and egg counts were high in very young shrews. Infections were lost by late spring/early summer. Infection by hymenolepids did not show a clear seasonal pattern.

Table 6.3. Occurrence of eggs in faecal samples of *S.araneus* (see text for further explanation).

(a) *Choanotaenia crassiscolex*

Shrew no.	Time of Year					
	Jul 89	Sep 89	Dec 89	Feb 90	Apr 90	Jun 90
1	✓[1]	x[1]	x[1]	✓	✓	-
2	✓[1]	✓	✓	✓	x	x
3	-	x	x[1]	✓[1]	x	x
4	-	✓	x	-	-	x
5	-	✓	x[1]	x	x	-

(b) *Hymenolepis schaldybini*

Shrew no.	Time of Year					
	Jul 89	Sep 89	Dec 89	Feb 90	Apr 90	Jun 90
1	✓[1]	x[1]	x[1]	✓	✓	-
2	x[1]	✓	x	✓	x	x
3	-	x	x[1]	x[1]	x	x
4	-	x	x	-	-	x
5	-	x	x[1]	x	x	-

(c) *Hymenolepis furcata*

Shrew no.	Time of Year					
	Jul 89	Sep 89	Dec 89	Feb 90	Apr 90	Jun 90
1	x[1]	x[1]	x[1]	x	x	-
2	x[1]	x	x	x	x	x
3	-	✓	x[1]	x[1]	x	x
4	-	✓	x	-	-	x
5	-	x	x[1]	✓	x	-

Table 6.4. The occurrence of eggs of *Longistriata spp.* and *Parastrongyloides winchesi* in faecal samples of *S.araneus*.

Shrew no.	Time of Year					
	Jul 89	Sep 89	Dec 89	Feb 90	Apr 90	Jun 90
1	✓[1]	✓[1]	✓[1]	✓	✓	-
2	x[1]	✓	x	✓	✓	✓
3	-	✓	x[1]	x[1]	✓	x
4	-	x	x	-	-	✓
5	-	✓	x[1]	x	x	-

Nematodes

Infection by *Longistriata spp. / Parastrongyloides winchesi* showed a similar pattern to the autopsy results, with prevalence and egg counts increasing over the summer, dropping in the winter and increasing again in the spring and summer. Capillariinids could not be detected in the autumn/winter, but were present in the spring and summer.

Digeneans

Very few digenean eggs were detected.

The faecal egg count results demonstrated that infections could be lost at one time of year and regained later on.

Variation in Helminth Abundance Relative to Habitat

Sufficient specimens of *S.araneus* were trapped to enable Cranbourne Chase, Lipper Pond and Silwood Park to be compared; for *S.minutus*, only Cranbourne Chase and Lipper Pond could be compared.

Cestodes

All cestode species were present at Cranbourne Chase; three of the less common species (*Choanotaenia hepatica, Hymenolepis prolifer* and *H.jacutensis*) were absent from Lipper Pond; four species (*Choanotaenia hepatica, Hymenolepis prolifer, H.diaphana* and *H.infirma*) were absent from Silwood Park.

Prevalence of infection by *Choanotaenia crassiscolex* was similar at all three sites, but intensity of infection at Silwood (21.2) was significantly greater ($0.01<P<0.05$) than at the other two sites (8.7 at Cranbourne and 9.7 at Lipper).

Prevalence and intensity of hymenolepids was much greater at Cranbourne than at either of the other two sites, although intensity of infection by *H.scutigera* was highest at Silwood Park (not statistically significant). For *H.schaldybini* in *S.araneus* the intensity at Cranbourne (36.8) was significantly greater than at Lipper (25.3, $0.01<P<0.05$) and Silwood (10.9, $0.001<P<0.01$); the prevalence at Cranbourne (78%) was greater than at Lipper (62%, not statistically significant) and Silwood (33%, $P<0.001$).

Nematodes

With the exception of *Stammerinema soricis* which was only found at Silwood, and the unidentified nematode larvae which were absent from Silwood, all nematode species were found at each site.

In *S.araneus, Porrocaecum sp.* was more prevalent (86%) at Lipper than at Silwood (67%) and Cranbourne (71%). The intensity of infection was also highest at Lipper (22.3, compared to 10.4 at Silwood and 5.7 at Cranbourne). The intensity of infection by this parasite was significantly greater at Lipper than at Cranbourne ($P<0.001$). Infections in *S.minutus* were too small for any site differences to be significant.

Prevalences of *Longistriata spp.* were similar at all sites and in both species of shrews, but intensity of infection in both host species was much greater at Lipper than at Cranbourne ($0.01<P<0.05$ for both host species). In *S.araneus* the intensity of infection at Silwood and Cranbourne was very similar.

The intensity of infection of *S.minutus* by *Stefanskostrongylus soricis* was similar at both sites (6.82 at Cranbourne and 6.0 Lipper) but the prevalence at Cranbourne

(70%) was significantly greater ($P<0.001$) than at Lipper (13%).

Eucoleus kutori was not found in *S.minutus* at Lipper, but was found in 49% of *S.minutus* at Cranbourne. Numbers of *E.kutori* in *S.araneus* were too low to be of any importance.

Digeneans and Acanthocephalans

The two species of digeneans and the acanthocephalan species, *Gordiorhynchus aluconis*, were more prevalent and found at greater intensities at Lipper than at Cranbourne. The prevalence and intensity of infection of *S.araneus* by *Brachylaemus fulvus* at Lipper (73%, 6.2) were significantly greater than at Cranbourne (51%, 2.4) ($0.01<P<0.05$ in both cases). The prevalence of infection of *S.minutus* by *Dicrocoelium soricis* was significantly greater ($P<0.001$) at Lipper (48%) than at Cranbourne (6%).

The only specimens of *Dicrocoelium soricis* recovered from *S.araneus* were from specimens trapped at Silwood. Prevalences and intensities of *Brachylaemus fulvus* at Lipper and Silwood were not significantly different.

The prevalence of infection by *G.aluconis* was significantly greater ($0.001<P<0.01$) at Lipper ($P_{sa}=62\%$, $P_{sm}=74\%$) than at Cranbourne ($P_{sa}=27\%$, $P_{sm}=34\%$) in both host species and intensity of infection of *S.minutus* was significantly greater ($0.01<P<0.05$) at Lipper (17.4) than at Cranbourne (5.3).

Ectoparasites

Fleas were more abundant at Cranbourne on both host species; for *S.araneus* the intensity at Cranbourne (2.8) was significantly greater ($0.01<P<0.05$) than at Lipper (1.6). Ticks were more abundant on *S.minutus* at Cranbourne while on *S.araneus* they were slightly more abundant at Lipper.

In general the abundance of each helminth species was more similar between Lipper and Silwood than between Cranbourne and Silwood or Cranbourne and Lipper.

Other Sites

Three helminth species appeared to be relatively common at some sites although absent or rare at the main study sites.

Three out of five *S.minutus* from "Alderhurst" were infected with *Stammerinema soricis.*

Three out of four *S.araneus* from "Huntersdale" were infected with *Choanotaenia hepatica* and six out of twelve *S.araneus* from Dungeness were infected with *Opisthioglyphe sobolevi.*

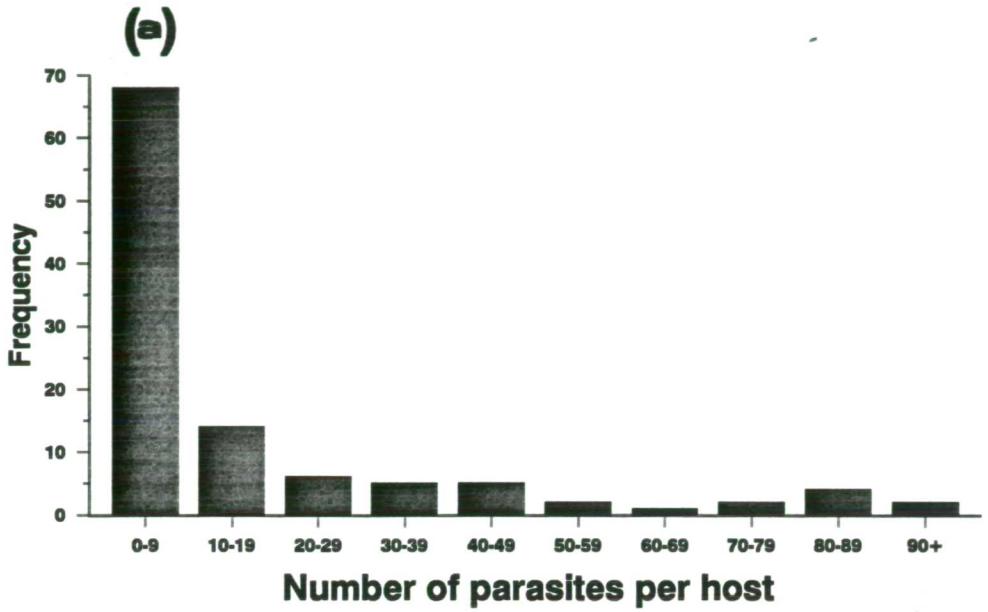
Frequency Distribution of Helminths

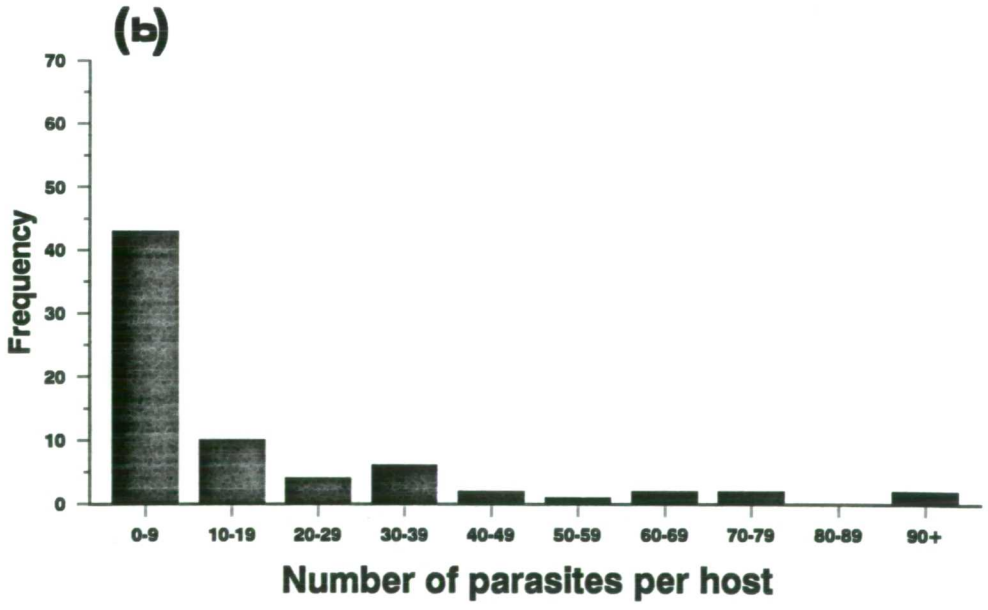
All helminth parasites were found to be overdispersed within the host population. The variance/mean values for the most abundant parasites are as follows: *Hymenolepis schaldybini* in *S.araneus* 100.6, 43.3 in *S.minutus; H.scutigera* in *S.araneus* 65.9; *Choanotaenia crassiscolex* in *S.araneus* 17.2; *Longistriata didas* in *S.araneus* 42.3, 13.3 in *S.minutus; Gordiorhynchus aluconis* in *S.minutus* 29.1.

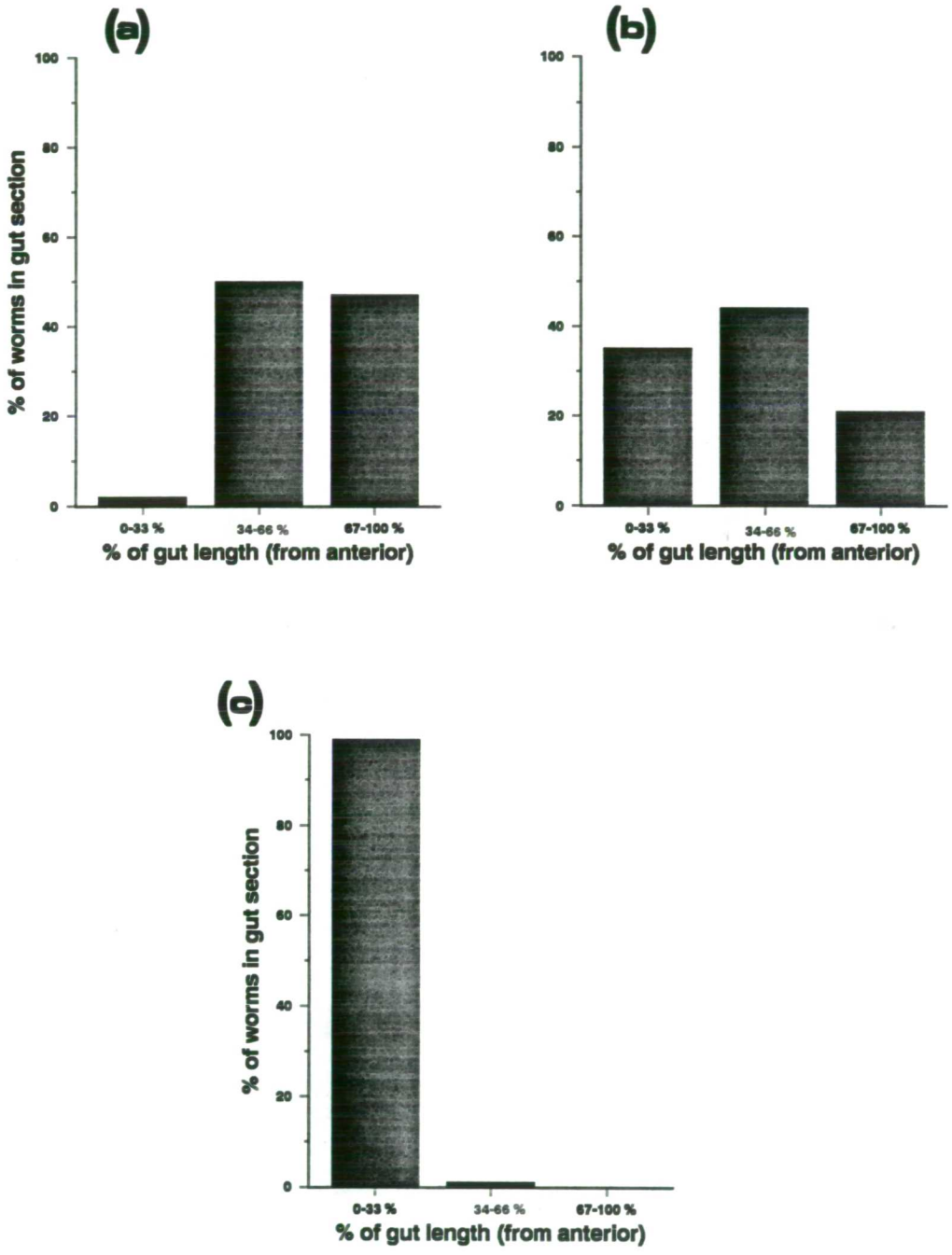
The frequency distribution of *H.schaldybini* in *S.araneus* and *S.minutus* is shown in Figure 6.5.

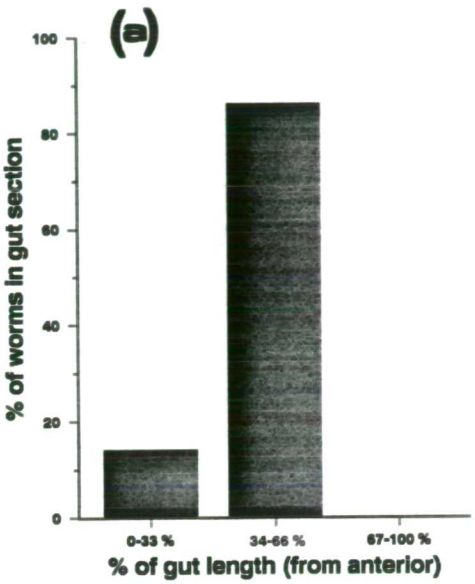
Niche Separation of Intestinal Helminths

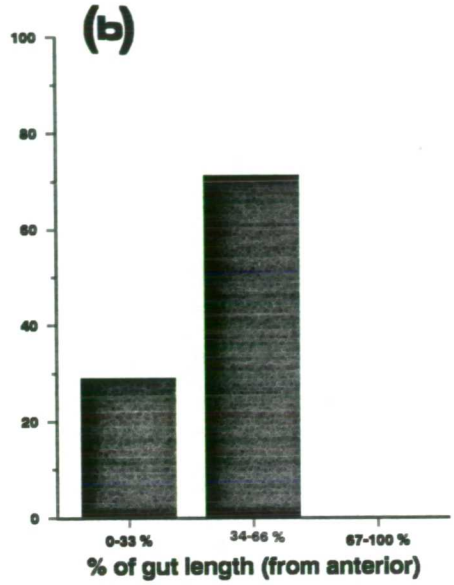
The intestines of 36 *S.araneus* and 31 *S.minutus* were divided into three equal sections and the numbers of each helminth species present in each section counted. The results for those helminths present in appreciable numbers are shown in Table 6.5 and Figures 6.6, to 6.10. The results demonstrate niche separation: *Choanotaenia crassiscolex* was found almost exclusively in the anterior section of the gut; *Hymenolepis schaldybini* was found mainly in the anterior and middle gut sections in *S.minutus* and mainly in the middle and posterior gut sections in *S.araneus; H.furcata* was found mainly in the middle gut section in both species and *H.scutigera* mainly in the middle and posterior sections. Nematodes

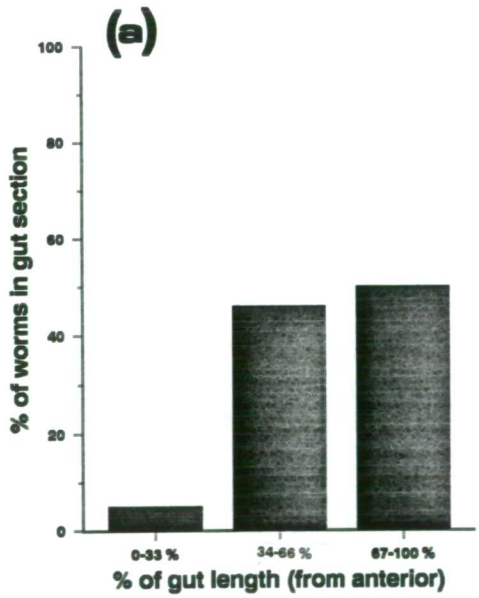

(a)
Frequency
70
60
50
40
30
20
10
0
0-9
10-19
20-29
30-39
40-49
50-59
60-69
70-79
80-89
90+
Number of parasites per host

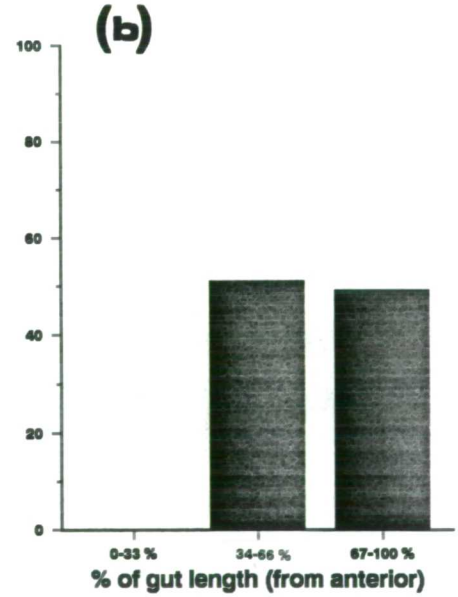

(b)
Frequency
70
60
50
40
30
20
10
0
0-9
10-19
20-29
30-39
40-49
50-59
60-69
70-79
80-89
90+
Number of parasites per host

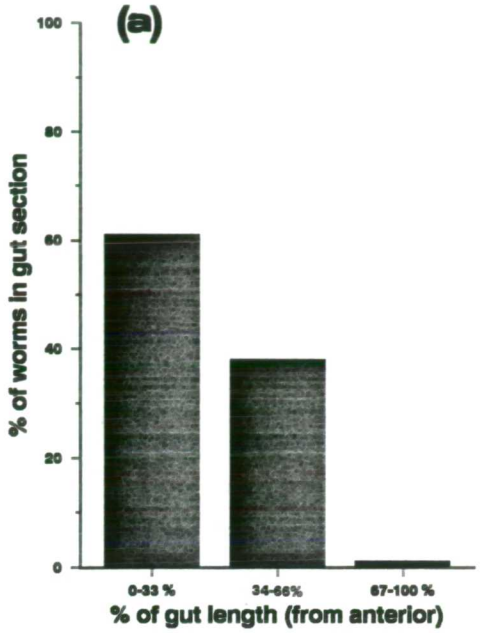

(a)
100
80
60
40
20
0
% of worms in gut section
0-33 %
34-66 %
67-100 %
% of gut length (from anterior)
(b)
100
80
60
40
20
0
0-33 %
34-66 %
67-100 %
% of gut length (from anterior)
(c)
100
80
60
40
20
0
% of worms in gut section
0-33 %
34-66 %
67-100 %
% of gut length (from anterior)

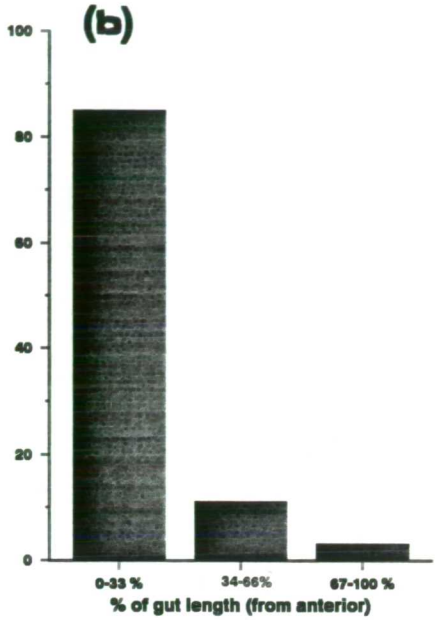

(a)
100
80
60
40
20
0
% of worms in gut section
0-33 %
34-66 %
67-100 %
% of gut length (from anterior)

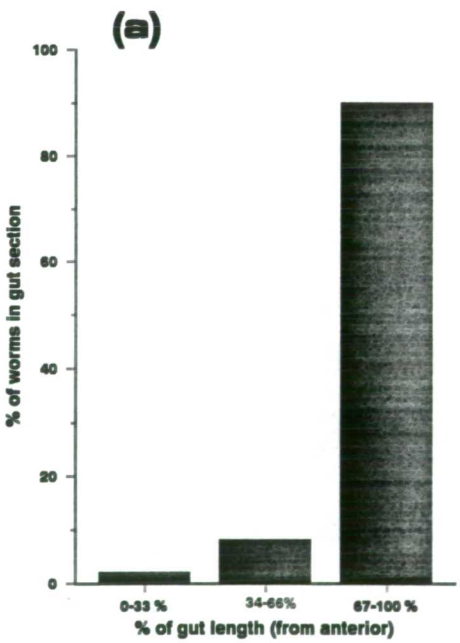

(b)
100
80
60
40
20
0
0-33 %
34-66 %
67-100 %
% of gut length (from anterior)

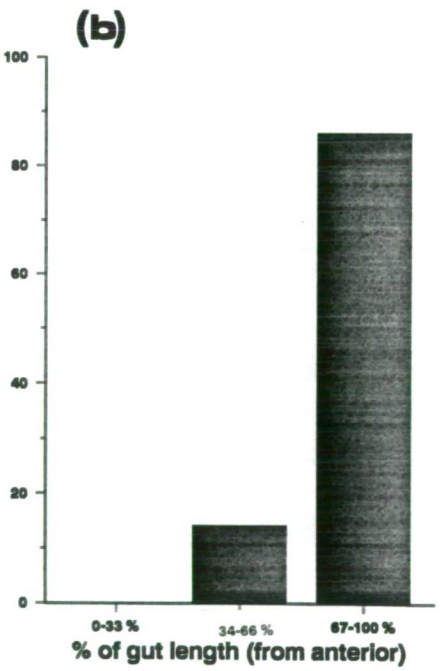

(a)
100
80
60
40
20
0
% of worms in gut section
0-33 %
34-66 %
67-100 %
% of gut length (from anterior)


(b)
100
80
60
40
20
0
0-33 %
34-66 %
67-100 %
% of gut length (from anterior)


(a)
% of worms in gut section
100
80
60
40
20
0
0-33 %
34-66%
67-100 %
% of gut length (from anterior)


(b)
100
80
60
40
20
0
0-33 %
34-66%
67-100 %
% of gut length (from anterior)


(a)
% of worms in gut section
100
80
60
40
20
0
0-33 %
34-66%
67-100 %
% of gut length (from anterior)


(b)
100
80
60
40
20
0
0-33 %
34-66 %
67-100 %
% of gut length (from anterior)

of *Longistriata spp.* were found mainly in the anterior and middle sections while the majority of specimens of *Parastrongyloides winchesi* occupied the posterior gut section.

Table 6.5. Niche separation of intestinal helminths in *Sorex araneus* and *S.minutus*.

Parasite	*S.araneus*			*S.minutus*		
	Gut Section					
	1	2	3	1	2	3
Choanotaenia crassiscolex	***	x	x	***	x	x
Hymenolepis schaldybini	x	**	**	**	**	*
Hymenolepis scutigera	x	**	**	x	**	**
Hymenolepis furcata	*	***	x	*	**	x
Longistriata spp.	***	**	x	***	*	x
Parastrongyloides winchesi	x	*	***	x	*	***

x = 0-5% of parasites in this gut section

* = 6-30% of parasites found in this gut section

** = 31-60% of parasites

*** = 61-100% of parasites

<u>Possible Impact of Helminths on the Shrew Host</u>

Infections by the cestode, *Choanotaenia hepatica* caused a clearly visible swelling of the bile duct and *Stammerinema soricis* caused a nodule to be formed in the stomach wall in which the anterior end was embedded.

Apart from the above effects there were no obvious differences in physical appearances between non-parasitised and parasitised individuals.

The effect of helminth parasites on the two shrew species was assessed by

relating the number of parasites present and the size of the spleen and the pancreas of Ascelli of individual hosts.

The autopsy results for the shrews of each species were divided into the following groups: October/November, December/January, March, May (*S.minutus* only), Juveniles in June-September and Adults in June-September. These groupings were used in order to eliminate the seasonal changes known to occur in the weights of the spleen and pancreas of Ascelli (Twigg & Hughes, 1970).

For each of the above groups of shrews, Kendall's Rank-Correlation Coefficient, K was calculated for the relationship between spleen weight and worm burden, and pancreas of Ascelli weight and worm burden for the most abundant parasites (Tables 6.6, 6.7). A negative value of K denotes that there was a negative correlation between the two parameters tested, e.g. the figure '3' in the first column and first row of Table 6.6 indicates that for three of the groups of shrews the weight of the spleen decreased as the worm burden of *Choanotaenia crassiscolex* increased. A positive value of K denotes that there was a positive correlation between the two parameters tested, and a zero value indicates that there was no correlation whatsoever. The number of groups in which each type of K value was found is listed in the table with the number of these values which were statistically significant indicated in brackets.

There was no obvious pattern of correlation between worm burden and size of either the spleen or the pancreas of Ascelli.

Table 6.6. Values of Kendall's Rank-Correlation Coefficient, K for the relationship between weight of spleen and pancreas of Ascelli against worm burden in *Sorex araneus*. For further explanation see text.

Organ/Parasite tested	Negative values of K	Positive values of K	Zero values of K
Spleen wt., *C.crassiscolex*	3	2 (1 significant)	0
P. of Ascelli, *C.crassiscolex*	2	3	0
Spleen, *H.schaldybini*	3	2	0
P. of Ascelli, *H.schaldybini*	3	2 (1 significant)	0
Spleen, *H.scutigera*	2	2 (1 significant)	1
P. of Ascelli, *H.scutigera*	2	3 (1 significant)	0
Spleen, *Longistriata spp.*	1 (significant)	4 (2 significant)	0
P. of Ascelli, *Longistriata spp.*	1	4	0
Spleen, *Porrocaecum sp.*	0	3	2
P. of Ascelli, *Porrocaecum sp.*	1	2 (1 significant)	2

Table 6.7. Values of Kendall's Rank-Correlation Coefficient, K for the relationship between weight of spleen and pancreas of Ascelli against worm burden in *Sorex minutus*.

Values Tested	Negative values of K	Positive values of K	Zero values of K
Spleen, *H.schaldybini*	2	4	0
P. of Ascelli, *H.schaldybini*	1	3 (1 significant)	1
Spleen, *Longistriata spp.*	3 (1 significant)	2	0
P. of Ascelli, *Longistriata spp.*	2	4 (1 significant)	0
Spleen, *G.aluconis*	4 (1 significant)	0	0
P. of Ascelli, *G.aluconis*	1 (significant)	2	1

Discussion

Prevalences and Intensities of infection in *Sorex araneus*

Almost all the shrews examined in the present study were infected suggesting that most of the host population had the potential to be regulated by helminth parasites. The following species of helminths were present at significant prevalences and intensities: *Choanotaenia crassiscolex* (prevalence 71%, intensity 11.0), *Hymenolepis schaldybini* (61%, 30.1), *H.scutigera* (58%, 26.7); *Brachylaemus fulvus* (62%, 4.3), *Porrocaecum spp.* (74%, 13.0), *Longistriata spp.* (81%, 19.6) - all species of *Longistriata* are likely to cause similar pathological effects so they may be grouped together for the purpose of examining their impact on the shrew population.

In general the values of prevalence and intensity differ from those found by other authors who investigated the helminths of *S.araneus*. This was especially noticeable when examining the work of Soltys (1952), Prokopič (1959), Prokopič et al. (1974) and Haukisalmi (1989) where the prevalence and intensity values were sometimes 10-20 times smaller than those obtained in the present study. The above discrepancies are difficult to explain, but even in studies where the prevalence and intensity values were quite similar to those obtained in the present study (Kisielewska, 1961; Lewis, 1968; Prokopič 1970a; Prokopič 1970b) the values tended to be less than those obtained in the present study. Thus it appears that the host populations studied in the present investigation harboured above average levels of infection.

Host Age

Cestodes

The higher intensities found in the juveniles suggest less resistance to infection or that the juveniles are eating food more likely to contain infective stages. The latter explanation seems unlikely as Churchfield (personal communication) found no apparent difference in the diets of adult and juvenile shrews during the

summer period. Kisielewska (1961) found that the prevalence of infection was slightly greater in adult than in juvenile *S.araneus* and attributed this to the continual recruitment of uninfected juveniles into the population. No such difference was found in the present study as every shrew except three was infected with cestodes.

Nematodes

The significantly greater numbers of *Porrocaecum* in adult *S.araneus* (also observed by Erkinaro & Heikura, 1977) are readily explained by the fact that adults have had a longer period over which to accumulate the parasite. The very low intensity of infection in the juveniles make it unlikely that *Porrocaecum* infections could cause their mortality in the autumn as suggested by Buckner (1969).

The other differences in nematode fauna are not so readily explained, but might be a combination of the longer period of time over which the adults have been able to accumulate infective stages and differences in feeding habits between adult and juvenile shrews.

Acanthocephalans

The higher levels of infection of *Gordiorhynchus aluconis* in adults are likely to be due to the longer time of exposure to infective stages.

Host Sex

In other host-parasite systems worm burdens in male hosts have been found to be greater than in females and this has been attributed to hormonal effects (Beck, 1952; Solomon, 1969). Kisielewska (1961) found that male *S.araneus* harboured more cestodes than females, but did not state whether this difference was statistically significant. Erkinaro & Heikura (1977) found that male *S.araneus* harboured significantly more *Porrocaecum sp.* than females. These differences were not found in the present study, but this may have been due to the fairly small sample sizes used (17 males, 19 females).

Prevalences and Intensities of infection in *Sorex minutus*

As in the case of *S.araneus*, prevalences and intensities were high enough to give helminths the potential to have a significant affect on the host population, this was especially true of *Hymenolepis schaldybini* (prevalence 68%, intensity 24.5), *Stefanskostrongylus soricis* (50%, 6.8), *Longistriata spp.* (83%, 8.6) and *Gordiorhynchus aluconis* (46%, 11.5).

The differences in prevalence and intensity values between those found in the present study and those recorded by other authors were similar to those described above for *S.araneus*. Grainger & Fairley (1978) found that in Ireland the prevalence and intensity of *G.aluconis* in *S.minutus* was much lower than that found in the present study. This may be attributed to the fact that the tawny owl, the usual definitive host for this parasite, is not present in Ireland. The definitive host for *G.aluconis* in Ireland (assuming this was the parasite found by the above authors) is therefore unknown. A possible explanation for the lower values of prevalence and intensity recorded by Grainger & Fairley for some of the other parasites might be the absence from Ireland of *S.araneus* which may act as a reservoir host for the majority of helminths found in *S.minutus*.

Host Age

In contrast to *S.araneus*, no significant differences in cestode fauna were detected between adult and juvenile *S.minutus*. The comparable age-related differences in prevalences and intensities of nematodes and acanthocephalans to those found in *S.araneus* were presumably caused by the same factors.

The greater abundance of *Dicrocoelium soricis* in adult hosts may be due to the adults acquiring their infections before the birth of the juveniles. The intermediate host for *D.soricis* may not have been available later in the year when both adult and juvenile shrews were present.

Host Sex

Although there was little difference in infection between male and female

shrews it is interesting to note that the one statistically significant difference involved a higher level of infection in male shrews. As discussed above, male hosts are often more heavily infected than females.

Comparison of Infection Levels in *Sorex araneus* and *S.minutus*

The general principle that the prevalence and intensity of a given helminth species is higher in *S.araneus* than in *S.minutus* is also evident from the work of Soltys (1952, 1954), Prokopič (1959), Lewis (1964, 1968) and Haukisalmi (1989). The latter author attributes this difference to the greater size of *S.araneus*; being larger, this species requires more food than *S.minutus* and will therefore come into contact with more infective stages of helminths. The larger intestine of *S.araneus* may also accommodate higher worm burdens.

The greater size of *S.araneus* provides an acceptable explanation for some of the differences in the helminth faunas of the two species. However, it does not account for all of the quantitative and qualitative differences in the helminth faunas of the two species.

The difference in prevalence and intensity of *H.scutigera* in the two host species may be explained by differences in availability of fleas (*Ctenopthalamus* and *Palaeopsylla* which are the intermediate host for this parasite (Prokopič, 1969; Smit, 1974, 1978). The prevalence of fleas in the present study was shown to be much greater on *S.araneus* than on *S.minutus*.

The considerably greater prevalences and intensities of *Choanotaenia crassiscolex* and *Brachylaemus fulvus* in *S.araneus* may be attributed to dietary differences between the two host species. In a study carried out at Silwood Park, Churchfield, Hollier & Brown (1991) found that gastropods were rarely eaten by *S.minutus* while they were a common prey item of *S.araneus*. In the present study larval stages of both *C.crassiscolex* and *B.fulvus* were found in gastropods (Chapter 5).

The prevalences and intensities of the following species are considerably higher in *S.araneus* than in *S.minutus*: *Porrocaecum sp.*, and the two capillariinids *Eucoleus oesophagicola* and *Liniscus incrassatus*. The prevalence and intensity values for *Parastrongyloides winchesi* were also significantly greater in *S.araneus*.

The main dietary difference between *S.araneus* and *S.minutus* is the large number of earthworms consumed by the former while they are rarely eaten by *S.minutus*. This suggests that earthworms may be the intermediate host for the above four species of nematode. Earthworms are known to be the intermediate host for the capillariinid species, *Aonotheca erinacei* (Rudolphi, 1819) Lopez-Neyra, 1947 (=*Capillaria erinacei*) found in another insectivore the hedgehog, *Erinaceus europaeus* Linnaeus, 1758 (Romashov, 1980). In addition to being found in the shrew, *P.winchesi* is also found in the mole, *Talpa europaea* Linnaeus, 1758 (Morgan, 1928) for whom earthworms are "the single most important prey" (Corbet & Harris, 1991). The fact that very few *Porrocaecum sp.* appear to be ingested during the summer period when earthworms are less abundant (Edwards & Lofty, 1977) is further evidence that earthworms may act as intermediate hosts for this species of nematode.

The following four helminth species are significantly more prevalent in *Sorex minutus* than in *S.araneus*: the digenean *Dicrocoelium soricis*, the capillariinid nematode *Eucoleus kutori*, the angiostrongylid nematode, *Stefanskostrongylus soricis* and the larva of the acanthocephalan, *Gordiorhynchus aluconis*. Lewis (1968) recorded similar interspecific differences in prevalence and intensity of *D.soricis* and *G.aluconis* to those found in the present study.

The intermediate hosts for the above four helminths are unknown, but it is suggested that they are invertebrates eaten in greater quantities by *S.minutus* than by *S.araneus*. Lewis (personal communication) found cercarial stages of a dicrocoeliid in *Discus sp.* (Gastropoda) from Rogate Field Centre, Petersfield, Hampshire, part of King's College, University of London. If this digenean was *D.soricis* it is likely that the cercarial stage penetrates an arthropod second intermediate host which is then eaten by the shrews; this arthropod is presumably eaten more often by *S.minutus* than by *S.araneus*.

The capillariinid *E.kutori* is unlikely to utilise earthworms as its intermediate host due to its scarcity in *S.araneus* and abundance in *S.minutus*.

Very few authors have discussed the possible reasons for the host-specificity of helminth parasites in shrews, but Prokopič (1959) and Kisielewska (1961)

considered that the ecology of the final hosts, particularly their feeding habits was an important factor. Vaucher (1971) considered that ecological differences contributed to inter-specific variation in worm burdens, but not to the qualitative differences in helminth species present.

Laboratory experiments would be required to determine whether differences in the prevalence and intensity of a given helminth in the two host species is due to differences in viability of the parasite in the two hosts.

Seasonal Variation in Infection Levels

Cestodes

The hymenolepid species followed a general trend of high worm burdens in the summer, which decreased to comparatively low levels during the winter, with the high levels of infection being regained in the following summer. There are two possible explanations for this phenomenon: either the heavily infected individuals died off in the autumn, decreasing the mean worm burden, or the majority of the cestodes were lost in the autumn and winter and were not adequately replaced during this period.

Previous studies on seasonal dynamics of helminth infections in *Sorex* shrews (Soltys, 1952, 1954; Kisielewska, 1961, 1964; Lewis 1964, 1968; Grainger & Fairley, 1978) have involved only the use of autopsy results so that the same individual shrews could not be examined at more than one point in time. However, in the present study faecal analysis data has shown that some shrews parasitised in the summer lose their parasites during the autumn/winter and regain them the following summer (Tables 6.3, 6.4). Kisielewska (1960a) found that wild-caught *S.araneus* were not parasitised by cestodes after they had been kept in the laboratory for six months, presumably because any tapeworms present initially had reached the end of their natural lifespan.

The seasonal trends in hymenolepid abundance were similar to those found by Soltys (1954), Kisielewska (1961) and Grainger & Fairley (1978). The trends in infection by *Hymenolepis schaldybini* can be explained by changes in availability of

their intermediate hosts (carabid beetles). Carabids are abundant in the summer, thus the juvenile shrews are able to acquire infections of *H.schaldybini*. However, in the autumn and winter very few carabids are available, thus cestodes lost from a host individual due to natural parasite mortality or host immune reactions, are not replaced. Carabid beetles become available as prey items in the spring and summer so the levels of infection are able to build up again.

The seasonal decline in infection levels of *H.scutigera* can be explained by the decrease in numbers of fleas (their intermediate host) during the winter months.

Nematodes

The gradual increase in abundance of *Porrocaecum sp.* during the lives of the hosts is consistent with the hypothesis that earthworms are the intermediate hosts as they would be readily available throughout the year.

The seasonal patterns of *Parastrongyloides winchesi* and *Longistriata spp.* infections obtained from the autopsy results were corroborated by the faecal analysis data. The low infection levels during the winter and high worm burdens in the summer suggest that the intermediate hosts of these species are arthropods present in large numbers in the summer. The higher temperatures in the summer might also favour the development of the eggs and any free-living larval stages present in the life-cycle.

Digeneans

The peaks in infection by digeneans occur just after the peak in number of gastropods (the intermediate hosts). Lewis (1968) also found a relationship between abundance of intermediate hosts of *Brachylaemus fulvus* and occurrence of the fluke in *S.araneus*.

Thus there was considerable seasonal variation in helminth infections of *S.araneus* and *S.minutus* which in some cases could be explained by changes in the availability of their intermediate hosts.

Variation in Helminth Abundance Relative to Habitat

The differences in total number of species found at the three main study sites can be attributed mainly to differences in the numbers of shrews examined. Thus the four least common species of cestodes were not found at Silwood Park, where only 22 shrews (21 *S.araneus* and one *S.minutus*) were obtained for autopsy, three of these cestode species were absent from Lipper Pond from where 60 shrews were autopsied (37 *S.araneus* and 23 *S.minutus*). But all cestode species were found at Cranbourne, where the sample size was 92 shrews (45 *S.araneus* and 47 *S.minutus*).

However, the absence of *Opisthioglyphe sobolevi* from the three main study sites cannot be explained in this way since 50% of a total of only twelve *S.araneus* from Dungeness were infected. Dungeness is a wetland area and the shrews trapped there were obtained from a thin band of vegetation (approximately 20-50m wide) around the edge of an Open Pit which is a naturally occurring body of water on a shingle substrate, surrounded by wetland vegetation. Open pits are unique to Dungeness. Pojmanska (1961) suggested that the intermediate hosts of *O.sobolevi* were a freshwater snail (first intermediate host) and the larva of a freshwater insect which metamorphoses on land (second intermediate host). This hypothesis is consistent with the high prevalence of *O.sobolevi* in the wetland habitat.

There is no obvious explanation for the higher prevalences of *Stammerinema soricis* and *Choanotaenia hepatica* at "Alderhurst" and "Huntersdale".

A general comparison of the helminth faunas of the shrews from the two grassland sites Lipper Pond and Silwood Park with Cranbourne Chase indicated several significant differences.

A possible explanation for the greater prevalence and intensity of hymenolepids at Cranbourne is as follows: the soil at Cranbourne has a higher organic content due to its peaty nature and this results in the presence of large numbers of small arthropods such as Collembola (Chapters 2, 5) which feed on decaying organic matter. These arthropods are likely to ingest hymenolepid eggs found in the shrew faeces. The shrews will become infected either through eating these arthropods or by consuming carabid beetles which prey on the arthropods.

The prevalence and intensity of *Choanotaenia crassiscolex* and the two digenean

species was largest at Silwood Park and smallest at Cranbourne Chase; this can be explained by differences in the abundance of gastropods (the intermediate hosts) at these sites. More gastropods were found at Silwood than at Lipper which in turn had more gastropods than Cranbourne.

The much larger numbers of *Porrocaecum sp.* found at Lipper and Silwood than at Cranbourne are consistent with the hypothesis that the intermediate host for this parasite is the earthworm. The peaty acid soil at Cranbourne is likely to support few earthworms (Edwards & Lofty, 1977).

The greater abundance of *G.aluconis* and *Longistriata spp.* at Lipper Pond than at Cranbourne and the greater abundance of *Stefanskostrongylus soricis* at Cranbourne than at Lipper may perhaps be due to differences in availability of intermediate hosts, but since the intermediate hosts of these species are unknown this must remain a tentative hypothesis. In the case of *S.soricis* a contributory factor to the greater intensity of infection at Cranbourne might be the greater density of *S.minutus* at this site as this larger host density may facilitate transmission.

The low number of *Eucoleus kutori* at Lipper compared to that at Cranbourne is further evidence that this species is carried by a different intermediate host than the other capillariinid species which are more abundant at Lipper.

Thus the habitat has an important influence on the nature of the helminth fauna found in *S.araneus* and *S.minutus* and this is likely to be due to habitat-dependent variation in the abundance of invertebrate intermediate hosts.

Prokopič (1957, 1959, 1970a) and Prokopič et al. (1974) also noticed variation in helminth fauna related to the habitat, but did not provide an explanation for this phenomenon.

The demonstration of habitat-dependent differences in helminth faunas illustrates the importance of examining hosts from a variety of habitats when conducting a helminthological survey.

Frequency Distribution of Helminths

The results of the present study clearly indicate that the helminth parasites of *S.araneus* and *S.minutus* are overdispersed in the host population. This overdispersion might be caused by aggregation of infective stages (Keymer & Anderson, 1979), as well as differences in susceptibility to parasitism caused by immunological differences (Wakelin, 1985, 1987), but it was not possible to investigate this in the present study.

Since the distribution of helminths was overdispersed some hosts had very large worm burdens which might have been able to exert a regulatory affect on the host population.

Niche Separation of Intestinal Helminths

Niche separation was evident in the present study. *Choanotaenia crassiscolex* was found almost exclusively in the first 33% of the intestine. *Hymenolepis schaldybini* was found throughout the length of the intestine, but appeared to have a preference for the anterior portion since in *S.minutus* where *C.crassiscolex* is absent *H.schaldybini* is found mainly in the first two intestinal sections, whereas in *S.araneus* it is virtually absent from the anterior third of the intestine. Thus it appears that competition between the two cestode species in *S.araneus* leads to a shift in the niche occupied by *H.schaldybini*. A similar scenario was observed under experimental conditions by Holmes (1961). He observed that when rats were infected with single-species infections of either *Moniliformis dubius* (Acanthocephala) or *Hymenolepis diminuta* (Cestoda) the helminths were generally found in the anterior third of the gut. In concurrent infections *Moniliformis dubius* tended to occupy the anterior third of the intestine while *Hymenolepis diminuta* was located in a more posterior position.

H.furcata was found mainly in the middle gut section, but once again a greater percentage of worms was found in the anterior section in *S.minutus* than in *S.araneus*, perhaps indicating competition with *C.crassiscolex* in *S.araneus*. *H.scutigera* was able to occupy successfully the posterior section of the intestine, a niche largely left vacant by the other cestode species.

Niche separation was also evident in the most common species of nematode present in the intestine. *Longistriata spp.* occupied the first two gut sections (especially the anterior one) while *Parastrongyloides winchesi* was found almost exclusively in the posterior intestinal section. The preference of members of the family Heligmosomatidae (to which *Longistriata* belongs) for the anterior region of the intestine was also observed in *Heligmosomoides polygyrus* (Lewis & Bryant, 1976; Keymer & Hiorns, 1986b).

Table 6.5 shows that the adaptation of different species of helminth to different regions of the intestine ensures a more optimal usage of this organ than if each species occupied the same niche. Further niche separation can be seen from Table 3.1 in Chapter 3, indicating the diversity of organs occupied by helminths. In the present study helminth species of *S.araneus* and *S.minutus* have been identified in the following organs: oesophagus, stomach, gall bladder, bile duct, liver, urinary bladder and lungs. Other authors working on the same host species have also located helminths in the kidneys (Jourdane, 1971) and spleen (Soltys, 1952).

Thus the helminths found in *S.araneus* and *S.minutus* are adapted to different niches or zootopes (Kisielewska, 1970) increasing the levels of parasitism which can be sustained by an individual host.

Possible Impact of Helminths on the Shrew Host

Any investigation of the impact of helminths on the pathology of a wild host population using field data alone is hampered by other factors which might influence the health and therefore the dynamics of the host population. Spratt (1990) lists the following factors contributing to regulation of host populations: predation, emigration, resource depletion and behavioral or physiological collapse.

In the present study the only direct effects observed were the swelling of the bile duct caused by *Choanotaenia hepatica* and the nodules on the stomach wall caused by *Stammerinema soricis*. The fact that parasites or at least their anterior ends, were embedded in the host tissues suggests that some damage would have been caused. The extent of the damage caused by cestodes of Soricidae was investigated by Vaucher (1971). He considered *Hymenolepis diaphana* and *H.infirma* to have no effect on the mucosa of the intestine. All the species with armed

scolices had some effect on the intestinal wall at the point of attachment. The effect varied depending on whether the worms were attached to the submucosa by only their rostellar hooks (as was the case with *H.schaldybini* and *H.singularis*) or by their suckers as well (*H.furcata*). Vaucher calculated that 70 specimens of *H.furcata* would be required to cause the destruction of 1% of the mucosa, while over 200 *H.schaldybini* would be required to cause the same amount of damage. Since such levels of infection were not found in the present study it can be deduced that the mechanical damage caused by cestodes was not significant. The same is likely to be true for the other species of helminths present.

Pathogenic effects are not limited to mechanical damage at the site of attachment. Helminths might compete with the host for food, produce inflammatory responses, cause a change in host behaviour or lead to a decrease in fecundity of the host.

Okhotina & Nadtochy (1970) found that *Mammanidula asperocutis*, a nematode found in the mammary glands of four species of *Sorex* (not *S.araneus* or *S.minutus*) prevented milk production, thereby leading to the death of the offspring. Yuill (1964) working on the gastrointestinal nematodes of cottontail rabbits (*Sylvilagus floridanus*) and Weatherly (1971) working on the larvae *Trichinella spiralis* (Nematoda) in swiss mice found that helminth infection reduced the number of progeny reared by the female hosts.

Hay et al. (1985) found that spontaneous running activity was increased in mice infected with larvae of the ascarid nematode *Toxocara canis*, although the infections had no obvious affect on the health of the mice. Such an increase in activity might increase the vulnerability of the host to predators and perhaps a similar effect is caused by the larvae of *Porrocaecum sp.* and *Gordiorhynchus aluconis* in shrews.

Ali & Behnke (1985) observed that *Nematospiroides dubius* (= *Heligmosomoides polygyrus*) infections in mice caused an enlargement of the spleen and the mesenteric lymph nodes (roughly equivalent to the pancreas of Ascelli in the present study). Enlargement of these organs indicates an increase in immunological activity.

Such an effect could not be demonstrated in the present study; the very few

cases of positive correlation between weight of lymphoid organ (spleen or pancreas of Ascelli) and worm burden might easily be the result of another factor. For example, in very young shrews both lymphoid organ weight and worm burden are likely to increase with age.

If any helminth had an appreciable effect on immunological activity then such an effect would be expected to be visible in all samples of shrews examined rather than in just one of the five or six samples as in the present study (Tables 6.6, 6.7).

The failure to demonstrate any serious pathogenic effects of helminths on *S.araneus* and *S.minutus* does not mean that such effects were absent; they could have been masked by other factors or just not have been detectable by the techniques employed in the present study.

Some cases of helminths increasing the mortality of small mammals have been demonstrated under laboratory conditions: Scott (1987) showed that *Heligmosomoides polygyrus* could regulate an experimental population of mice. Keymer & Hiorns (1986b) also found evidence of parasite induced host mortality in mice infected with *H.polygyrus* and Fujita et al. (1990) found that *Taenia polyacantha* (Cestoda) caused mortality in some experimental host species.

The Role of Helminths in the Regulation of Shrew Populations

The two host species studied, *Sorex araneus* and *S.minutus*, are parasitised by a wide variety of helminths, some of which are present at high prevalences and intensities of infection. The degree of parasitism recorded in the populations investigated in the present study is higher than recorded by previous authors. Overdispersion of parasites in the host population leads to a small number of individuals having comparatively high worm burdens. These higher levels of parasitism do not appear, however, to cause sufficient mechanical damage to the host tissues to increase the mortality rate.

No relationship was found between worm burdens and the weights of secondary lymphoid organs so increased immunological activity as a result of helminth infection could not be demonstrated, although it may have occurred.

The decrease in intensity of infection during the autumn/winter was believed

by some authors to be indicative of mortality of heavily infected hosts. However, investigation of seasonal changes by the examination of faecal samples has shown this conclusion to be unlikely. The changes in worm burden with time are more likely to be due to fluctuations in the availability of infective stages caused by seasonal changes in abundance of invertebrate intermediate hosts.

Although parasitic helminths are not likely to be a major influence on the dynamics of the shrew population, they may be a contributory factor to host population changes. Helminths might cause a slight increase in host mortality rate, not detectable in the present study and might also produce behavioural changes which will increase vulnerability to predation. Keymer (1982) and Scott (1988) stated that parasitic infections act synergistically with poor nutrition or stress. Forrester (1971) found that lungworm infection was not usually fatal to bighorn sheep, but when combined with overcrowding, poor nutrition, inclement weather or multiple parasitism, it had a significant effect on mortality.

Under normal circumstances it would appear that parasites regulate their own populations thereby preventing the accumulation of lethal worm burdens. The results described in the niche separation section of this chapter suggest that intestinal helminths were competing for attachment sites or nutritional substances in the present study. If there was density-dependent competition between helminths it follows that the larger host, *S.araneus* would be able to support more helminths than its smaller counterpart and this was indeed the case in the present study.

Hesselberg & Andreassen (1975), Jones & Tan (1971), Halvorsen & Andersen (1974) and Roberts (1961) showed that the rate of growth and metabolism of cestodes in small mammals was dependent on the number of worms present in the intestine. Chandler (1939) found that *Hymenolepis diminuta* were smaller if their density in the intestine of rats was increased. The rate of establishment and growth in secondary infections was not as great as in primary infections. The effect was apparently due to 'crowding' (or some other type of competition between helminths) since when the primary infection was removed mechanically, normal secondary levels of infection were established.

Read (1951) suggested that oxygen supply might be a factor limiting growth

of helminth populations in the intestine.

Thus helminth parasites do not appear to be responsible for the autumnal decline of *Sorex araneus* and *S.minutus*, but it is possible that they may, under certain circumstances have a limited effect on host mortality.

Chapter 7
General Discussion.

General Discussion

Studies of shrew populations have been carried out at three sites (Chapter 2). Use of a mark-release-recapture technique at Silwood Park demonstrated that the population cycle of *Sorex araneus* at this site followed a similar pattern to that observed by earlier workers. The population size at Silwood increased considerably during the summer as juveniles were recruited into the population. The summer peak was followed by an autumnal decline in numbers involving all the adults and a significant proportion of the juveniles. The size of the population remained relatively stable during the winter and spring. The apparent increase in population size between February and April when no juveniles were being recruited into the population could be attributed to an increase in activity of the shrews (Gebczynski, 1965; Buchalczyk, 1972) and to a greater amount of time spent above ground than during the winter (Michielsen, 1966; Churchfield, 1979). The increase in activity involved a more nomadic behaviour in the males ranging over large areas in search of mates. A review of the literature suggests that the autumnal die-off of adult shrews is due to old age exacerbated by the stress of territorial interactions. Such stresses might also play an important part in the death of a proportion of the juveniles.

The regular removal of *Sorex araneus* and *S.minutus* from the Cranbourne and Lipper study sites did not allow the population cycles to be studied accurately, but the trends in numbers of captures were similar to those at Silwood and the Silwood population could therefore be taken as a model of the population changes taking place at the other two sites.

The greater numbers of *S.minutus* at the peaty Cranbourne site compared to the sandy loam grassland site at Lipper was consistent with the greater numbers of *S.minutus* found on peaty sites by other authors (Grainger & Fairley, 1978; Butterfield et al., 1981; Yalden, 1981). These results might be due to the greater abundance of small arthropods, more suitable to *S.minutus* at Cranbourne.

The helminth populations at Cranbourne and Lipper were studied in detail by the autopsy of shrews trapped at regular intervals. Information on the helminths present at Silwood was obtained mainly from the examination of faecal samples

and also by autopsy of a few specimens.

Further helminth material was obtained from sites in Buckinghamshire, Kent, Oxfordshire and Surrey. The present study of the helminths of *S.araneus* and *S.minutus* is one of few comprehensive studies carried out in Britain. Several helminths were recorded from Britain for the first time, namely the cestodes, *Hymenolepis infirma, H.jacutensis, H.prolifer* and *H.schaldybini,* the nematode, *Stefanskostrongylus soricis,* and the digenean *Opisthioglyphe sobolevi. S.minutus* is a new host for *Eucoleus kutori.*

A smaller number of helminths (30 species) have now been identified from *S.araneus* and *S.minutus* in Britain than from these species in mainland Europe (45 species). Further work is necessary to establish whether this is a true reflection of the number of species present in Britain or whether an examination of a larger number of shrews from a greater diversity of habitats will reveal the presence of more helminth species.

Few descriptive studies have previously been carried out on helminths of British shrews, thus the morphological data from the present study will provide a useful source of reference for future work on geographical variation in the morphology of these helminths.

Several improvements have been made to the existing descriptions of the helminths examined in the present study. The relative positions of the end of the oesophagus and the excretory pore of the nematode, *Stefanskostrongylus soricis,* have been determined by observing living specimens. The ultrastructure of the cephalic region and cuticle of *S.soricis* have been described using SEM. Detailed morphometric data have been recorded on the nematode, *Parastrongyloides winchesi,* from the shrew; the only previous description of this parasite concerned specimens from the mole. Two species of nematode larvae have been described from *S.araneus* for the first time, one of them also being recovered from *S.minutus.* Further work is required to establish their identity. The number of rostellar hooks of the cestode, *Choanotaenia hepatica,* has been found to be 46 in agreement with Baer (1932) and at variance with Zarnowski (1955). The dimensions of the testes of the digenean, *Opisthioglyphe sobolevi,* were recorded for the first time.

Using SEM, the identity of the acanthella stages of the acanthocephalan,

Gordiorhynchus aluconis, has been established by comparison with adult specimens from the tawny owl, *Strix aluco*. The taxonomy of *G.aluconis*, *Opisthioglyphe sobolevi* and *Stefanskostrongylus soricis* has also been discussed.

The nematodes, *Longistriata didas* Thomas 1953 and *L.pseudodidas* Vaucher & Durette-Desset 1973 have been synonymised and the cestodes *Hymenolepis schaldybini* and *H.singularis* have been shown to be separate species rather than a single species as suggested by Zarnowski (1955).

Further work is required on the taxonomy of many of the helminths especially on the nematodes of the genus *Longistriata*.

The literature on helminths of British and mainland European *S.araneus* and *S.minutus* has been reviewed and reasons suggested for likely cases of incorrect identification. Up-to-date lists of the helminths recorded from *S.araneus* and *S.minutus* in Europe have been compiled.

Data on the morphology of the helminth eggs, obtained from autopsies of shrews from Windsor Great Park, allowed the eggs to be identified from the faeces of shrews without necessitating culling of the host. All helminth eggs apart from those of *Longistriata spp.* and *Parastrongyloides winchesi* could be identified to species.

In order to identify and count the eggs present in faecal samples it was necessary to develop a new faecal analysis technique. The technique developed was a modification of the dilution count method (Stoll, 1923). This method was much more efficient than the commonly used McMaster technique in terms of the percentage of eggs recovered; this, in conjunction with results obtained by Abu-Madi (unpublished) for eggs of *Heligmosomoides polygyrus* (Nematoda) in laboratory mice, suggests that the effectiveness of the McMaster technique should be re-evaluated.

Laboratory experiments indicated that there was no correlation between EPG or EPD and number of mature worms present. This is in agreement with the work of Watkins & Harvey (1942) on the silver fox and Richards (1991) on the red fox. Thus it is not possible to estimate worm burdens from faecal egg counts.

Laboratory experiments also confirmed that there was no periodicity in egg output and that in fact the numbers of eggs produced fluctuated erratically. This

fluctuation in egg output may be one of the reasons for the lack of correlation between faecal egg counts and number of mature worms. Other factors responsible for this might be intraspecific competition between helminths (Jones & Tan, 1971, Kerboeuf, 1982), interspecific competition (Holmes, 1961), differences in egg output related to helminth age (Chai et al., 1981, Richards, 1991) and the effects of host hormones (Beck, 1952) or antibodies (Keymer & Hiorns, 1986b).

Examination of invertebrates pitfall-trapped at the three main study sites revealed the presence of larvae of some of the helminth species recovered from *Sorex araneus* and *S.minutus*. Cysticerci of *Choanotaenia crassiscolex* were recovered from the gastropod snail *Vitrina pellucida* and cysticerci of *Hymenolepis schaldybini* were found in the staphylinid beetle *Anthobium unicolor*. *Vitrina pellucida* is known to feed on organic matter (P.Newell, personal communication) and may therefore become infected through feeding on the faeces of shrews. Gastropods are an important feature in the diet of *S.araneus* and fragments of the shell of *V.pellucida* have been found in Longworth traps occupied by *S.araneus*. Thus *V.pellucida* is an effective intermediate host for *C.crassiscolex*. *Anthobium unicolor* is a carnivorous beetle preying on small invertebrates such as collembolans, collembolans infected with cysticerci have been recorded by Prokopič (1968b), so *A.unicolor* may become infected through its food. *A.unicolor* is in the size range of beetle prey taken by *S.minutus* and *S.araneus* so infections will readily be passed on to the definitive host.

Metacercariae, probably belonging to the species, *Brachylaemus fulvus*, were recovered from the gastropod snails, *Oxychilus helveticus* and *V.pellucida*, both new intermediate hosts for this species. Both snail species prefer moist habitats (P.Newell, personal communication) which would be suitable for transmission of *B.fulvus*.

There is a paucity of information in the literature on the life cycles of helminth parasites of shrews, and where larval stages **have** been found in invertebrates, little attention has been paid to the possible pathways of transmission. In the present study, however, an attempt has been made to explain how the infected invertebrates might have acquired their infections, and the likelihood of these infections being passed on to the definitive host has been discussed. Experimental

infections, both of potential intermediate hosts using infective stages obtained from the shrew, and of the shrew, using infected intermediate hosts, are required to increase our knowledge of the life cycles.

The possible identity of some of the, as yet unknown, intermediate hosts may be inferred by a comparison of infection levels in *S.araneus* and *S.minutus*. Thus, the prevalences and intensities of infection by *Porrocaecum sp.*, *Eucoleus oesophagicola*, *Liniscus incrassatus* and *Parastrongyloides winchesi* are much higher in *S.araneus* than in *S.minutus* and it is suggested that this is because earthworms are the intermediate hosts since they are rarely eaten by *S.minutus*. It would therefore be desirable to examine earthworms from an infected area for the presence of these helminth species.

A knowledge of the identity of the intermediate hosts helps to explain the seasonal changes in infection observed. Thus the considerable increase in abundance of most helminth species during the spring and summer may be attributed to an increase in the availability of intermediate hosts and the drop in infection levels during the autumn is likely to be due to a decline in the intermediate host populations. In the present study, seasonal changes in abundance of the cestode, *Hymenolepis schaldybini*, could be correlated with seasonal changes in abundance of its intermediate hosts, carabid beetles; similar relationships were observed between *Hymenolepis scutigera* and fleas (Siphonaptera) and between *Brachylaemus fulvus* and gastropod snails (Chapter 6).

Analysis of faeces from the Silwood population of *S.araneus* demonstrated that some individuals lost their parasites during the autumn/winter and regained them during the following summer. The decrease in intensity of infection of shrews by helminth parasites in the autumn and winter, documented by several authors, can be explained by parasite mortality rather than by the death of the more heavily infected individuals; the parasites are not replaced by the ingestion of fresh larval stages as few intermediate hosts are available at this time of year (Chapter 6).

Preliminary results suggest that niche separation of intestinal helminths allows larger worm burdens to be supported. *Choanotaenia crassiscolex* is found in the anterior portion of the intestine of *S.araneus*, but is not present in *S.minutus*. The

percentage of individuals of *Hymenolepis schaldybini* in the anterior portion of the intestine of *S.minutus* is much greater than in *S.araneus*. Hence there appears to be competition for attachment sites or nutrients between these two helminth species. There was also evidence for niche separation in other intestinal helminths in the present study. Experimental evidence for niche separation and density-dependent limitation of growth of intestinal helminths has been recorded by Chandler (1939), Holmes (1961), Roberts (1961), Jones & Tan (1971), Halvorsen & Andersen (1974) and Hesselberg & Andreassen (1975). Thus the number of helminths present in the host may be limited by density-dependent factors and thus kept below a level which would be lethal to the host.

All helminth species studied were found to have an overdispersed distribution in the host population. This may have been due to aggregation of infective stages (Keymer & Anderson, 1979) and/or genetic differences in susceptibility to parasitism (Wakelin 1985, 1987). This overdispersion meant that some individual shrews harboured very high worm burdens. The pathogenic effects of parasites are generally related to the number of parasites present in the host (Anderson & May, 1978).

Vaucher (1971) calculated the numbers of intestinal cestodes necessary to cause appreciable mechanical damage to the host tissues, such worm burdens were not observed in the present study even in the heavily infected individuals. Ali & Behnke (1985), investigating the effects of the nematode, *Heligmosomoides polygyrus*, on laboratory mice, observed an enlargement of the spleen and mesenteric lymph nodes, indicating increased immunological activity. This was not observed in the present study nor were there any obvious differences in general appearance between heavily parasitised animals and those with low worm burdens.

There is therefore no evidence that helminths are a cause of mortality in *Sorex araneus* and *S.minutus* even in the present study where worm burdens were higher than recorded by previous authors. However, due to the multiplicity of factors operating in a field situation, the only way to adequately determine the effect of helminth parasites on the survival of shrews would be to carry out infection experiments on shrews maintained in the laboratory. This would be an extremely difficult operation because of the difficulty of obtaining infective stages of

helminths and of rearing shrews which are of sufficient similarity, genetically, to eliminate the effects of any genetic variation in susceptibility to helminth infection.

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